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CONTENTS

Observations on Streptomyces griseus. VI. EUGENE L. DULANEY 481
The effects of ionizing radiations from polonium on the spores of Aspergillus niger ..... SIGMUND BERK 488
The growth and metabolic behavior of Streptomyces venezuelae in liquid culture .. DAVID GOTTLIEB AND MARVIN LEGATOR 507
The utilization of carbon and nitrogen compounds by Ustilago zeae ..... FREDERICK T. WOLF 516
Comparisons of the survival of Aspergillus niger spores lyophilized by various methods R. H. HASKINS AND JOAN ANASTASIOU 523
The production and action of a tartrate-decomposing enzyme RICHARD R. BARTON 533
An undescribed fungus causing a root rot of red clover and other Leguminosae ..... J. W. GERDEMAN 548
Polyozellus multiplex and the family Phylacteriaceae ROKUYA IMAZUKI 555
On the status of the generic names Pyrenophora and Pleospora L. E. WEHMEYER 562
Pucciniostele, a genus of the rust fungi GEORGE B. CUMMINS AND M. J. THIRUMALACHAR 572
Two common lumber-staining fungi in the western United States ..... ROSS W. DAVIDSON 579
On Hyalodothis caricis ..... WILLIAM W. DIEHL 587
A new species of Monoblepharella ... FREDERICK K. SPARROW 592
Soil as a storage medium for fungi .. ALEXANDER BAKERSPIGEL 596
W. Lawrence White ..... W. G. H. SIU AND E. T. REESE 605
Notes and Brief Articles 613
Review 625

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VOL. XLV JULY–AUGUST, 1953 No. 4

OBSERVATIONS ON STREPTOMYCES GRISEUS. VI. FURTHER STUDIES ON STRAIN SELECTION FOR IMPROVED STREPTOMYCIN PRODUCTION [1]

EUGENE L. DULANEY

(WITH 1 FIGURE)

The use of induced mutation and strain selection to improve yields of a product produced by a microorganism is now well recognized. The marked increase in penicillin production that resulted from the application of this approach has been well documented (**1**, **4**). Thus far, however, nothing has been published concerning the use of this method to improve yields of other antibiotics with the exception of streptomycin, and even in this instance, the data are meager.

Stanley (**6**) used strain selection to improve streptomycin yields and reported maximum broth potencies of 800 γ/ml. Savage (**5**) reported the isolation of strains that produced activities of 1,000 γ/ml, but noted their marked instability. Dulaney, Ruger, and Hlavac (**2**) published on the isolation of mutants capable of producing 320% more streptomycin than the starting culture.

[1] Contribution from the research laboratories of Merck & Co., Inc., Rahway, New Jersey.

[MYCOLOGIA for May–June (**45**: 325–480) was issued June 22, 1953]

The present paper is a further report on our isolation of *Streptomyces griseus* mutants with an increased capacity for streptomycin production and presents scientific details of experiments which have led to the high-producing strain.

EXPERIMENTAL METHODS

The starting culture for this work was Waksman's No. 4 isolate of *Streptomyces griseus*. Stock cultures of this strain and of the mutants to be described later were prepared by drying spores in sterile soil. As cultures were needed, spores were transferred from the soil to slants of yeast extract-glucose agar or to an agar medium with a soybean meal-distillers solubles base. Changes in the medium used for developing spore crops were made necessary by the differences in sporulating capacities of the various strains. Spore crops were harvested by washing seven-day-old cultures incubated at 28° C with sterile, distilled water and filtering the resulting spore suspension through several layers of sterile absorbent cotton. This filtrate was subjected to various mutagenic agents, but only ultraviolet light and x-rays are pertinent to the results to be reported. In practice, exposure of the spores to the mutagens was for a time sufficient to kill approximately 99% of the spores. The treated suspensions were plated on a medium that supported good sporulation of the strain in question, and the plates were incubated at 28° C until good growth and sporulation occurred. Colonies thus developed were transferred to agar slants, incubated until sporulation was satisfactory, usually seven days, and either tested at once or stored at 4° C until used. It should be noted that there was no selection of the colonies for testing, but isolation of colonies was at random with all colonies on a plate usually being tested.

As work progressed, the experimental conditions used for screening the isolates changed. At first, a spore suspension was used as inoculum. This was later changed to vegetative growth developed on rotary shakers for 48 hours in a medium composed of glucose 1%, sodium chloride 1%, N-Z-amine 1%, meat extract 0.6%, and distilled water to volume. A number of other media would serve just as well. In all instances, however, one ml of

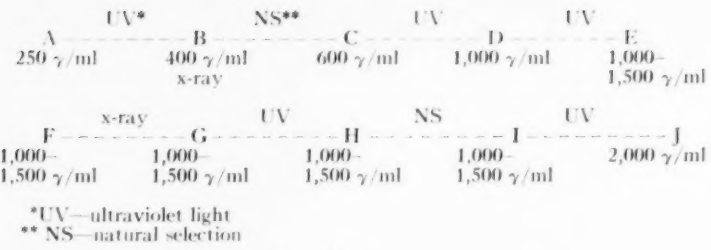
either spores or vegetative growth was used to inoculate a 40 ml volume of medium in either 125 ml or 250 ml Erlenmeyer flasks. The smaller flasks were used earlier in the work, but a change was made later to the larger size. These changes did not markedly affect the maximum broth potencies obtained but did somewhat change the time at which maximum activities occurred. The flasks were incubated at 28° C on rotary shakers moving at 220 rpm and describing a circle approximately one inch in diameter.

Whole broth samples, taken at appropriate intervals, were diluted in M/20 phosphate buffer and assayed by the cylinder-plate method using a reference standard of streptomycin sulfate or the calcium chloride complex.

With progressive isolation of improved strains, it was found to be necessary to change the concentration of some of the ingredients used in the screening medium. The medium used at the outset of these experiments, described in our earlier publication, was composed of soybean meal 2%, glucose 1%, sodium chloride 1%, and distilled water to volume. Following the isolation of each improved strain, it was found to be necessary to increase the level of nutrients in order to obtain maximum production. The subsequent use of a medium containing distillers solubles, as reported by McDaniel (3), resulted in improvements in the yield of streptomycin by the *S. griseus* cultures described in this paper. A medium used successfully for testing the high-producing mutants contained soybean meal 4%, glucose 2.5%, distillers solubles 0.5%, sodium chloride 0.25%, and distilled water to volume.

DISCUSSION OF RESULTS

A marked increase in streptomycin productivity has been accomplished by induced mutation and strain selection. Data obtained in laboratory experiments, illustrative of the type of improvements which can be demonstrated when working with small scale fermentations, are summarized in FIG. 1. The original culture, strain A, produced 250 γ/ml. This was a single colony isolate of the Waksman No. 4 strain. Since initiation of growth from spores was inhibited by streptomycin, it was made resistant to a streptomycin concentration of 600 γ/ml by serial passage in



*UV—ultraviolet light
** NS—natural selection

FIG. 1.

media containing increasing levels of streptomycin. It was feared that the sensitivity of the culture to streptomycin might be limiting the productivity of the culture. The study of development of resistance to streptomycin has been pursued no further, however, and cultures producing considerably in excess of 600 γ/ml have been obtained. If sensitivity to streptomycin has any significance in the isolation of superior strains, then we have been selecting for streptomycin resistance, as well as for streptomycin production. The testing procedures employed in the latter stages of this study, including the use of vegetative inoculum, are not conducive to the demonstration of streptomycin sensitivity in the mutant cultures.

Spores of strain A were subjected to ultraviolet light treatment, and strain B, capable of producing 60% more streptomycin than strain A, was thus obtained. In the beginning a great deal of exploratory work was concentrated on this culture which is also strain B of our previous publication (**2**). An isolate, strain C, which was 50% better than strain B and 140% better than strain A, was obtained from strain B by single-colony isolation. Strain C, strain G of our previous paper (**2**), was subjected to ultraviolet-light treatment, and strain D was obtained. This strain was listed as strain I in the previous publication (**2**) where it was reported as producing 3.2 times as much streptomycin as strain A. Actually it has been found that four times as much can be obtained by medium modification.

Strain D proved to be quite stable, but by induced mutation, markedly superior isolates were obtained from it. Strain E, which yielded 50% more streptomycin than strain D, was obtained from

strain D, following treatment with ultraviolet light. Strain F was obtained by subjecting strain E to x-radiation, and further x-radiation of strain F yielded strain G. By treatment of strain G with ultraviolet light, strain H was obtained, from which strain I was isolated by single-colony selection without treatment of the spores with a mutagen. All of these strains, namely, E, F, G, H, and I, were 50% improved over strain D, the highest producer described in previous publications. All the cultures were quite unstable, however, and after several transfers degenerated to production levels of their parent strain. This loss of productivity can best be ascribed to genetic instability or to heterocaryosis.

Strain I was subjected to treatment with ultraviolet light, and strain J was obtained. This culture is capable of producing relatively high yields of streptomycin and unlike its immediate progenitors is quite stable in relation to streptomycin productivity. Survival curves of this strain, following treatment with ultraviolet light, were somewhat sigmoid; whereas, survival curves of strains A and D were exponential. These sigmoid curves are indicative of multinucleate spores and indicate the possibility of heterocaryosis as the cause for the instability in strains E, F, G, H and I. It should be noted, however, that no stable high-producing homocaryotic races could be isolated from these strains by single-colony selection. In addition, if these strains were heterocaryons, their degeneration to a stable low production level indicates that the mutant nuclei were being selected against in the cytoplasm under the experimental conditions being used. Perhaps a change in the medium or in some other experimental factor would have induced a selection in favor of the mutant nuclei.

It should be emphasized that the values given in FIG. 1. were obtained through comparative tests under a given set of conditions. The most pertinent of these conditions was the use of the concentrated medium noted in the section on experimental methods. This point is emphasized, for some of these strains have been found to produce broth potencies different from those listed in FIG. 1 when tested with various fermentation media.

It should be realized that screening cultures under a given set

of conditions may result in cultures being selected that produce maximum broth potencies only under the screening conditions.

It is not always true, however, that a mutant capable of producing increased yields of a compound under the selecting conditions will produce its maximum yield only under those conditions. Hence, each successive mutant should be carefully studied in order to determine the conditions optimum for its productivity. For example, strain J is listed in FIG. 1 as producing eight times as much streptomycin as strain A. However, slight changes in such physical factors as aeration, agitation, and incubation temperature resulted in this strain producing a further increase in broth potencies of 15%.

The question arises as to the reason for increased productivity by these mutants. Perhaps increased productivity is due to a favorable change in some enzyme system operating in the synthesis of the limiting moiety of the streptomycin molecule. Perhaps this increased productivity is due to an enzymatic block that shunts intermediates from one path to the formation of streptomycin. It is also possible that the mutation results in the ability of the strain to overcome some inhibitor present in such a complex medium. In the successive selection of a number of mutants, a multiplicity of factors may be involved. Several of these mutants have been studied in synthetic media in an attempt to find physiological differences that might relate to the differences in productivity of streptomycin. The only difference found, however, was the ability of certain strains to grow efficiently and produce increased streptomycin broth potencies in a synthetic medium with L(-)proline as the sole nitrogen source. Strains B and C were markedly superior to strain A in such a medium, and strain D was far superior to strains C and B. None of the strains from E to J, however, were superior to strain D in the proline medium.

SUMMARY

Through the use of ultraviolet light, x-rays and natural selection, mutants were obtained that were capable of producing markedly higher streptomycin broth potencies than the starting culture. The highest streptomycin activities observed were over eight times

more than the broth potencies produced by the starting culture, Waksman No. 4, under the same experimental conditions. Many thousands of cultures were examined in the selection of cultures described in this paper.

The selectivity that often results from the experimental conditions used in screening cultures is emphasized.

MERCK & CO.,
RAHWAY, NEW JERSEY

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THE EFFECTS OF IONIZING RADIATIONS FROM POLONIUM ON THE SPORES OF ASPERGILLUS NIGER [1]

SIGMUND BERK [2]

(WITH 28 FIGURES)

The literature on the effects of ionizing radiations on fungi has recently been reviewed by Berk (**1952a, b**). More pertinent references will be listed in this article.

Biological effects are produced by many types of radiation if the dose is great enough. Of these, ionizing radiation is responsible for many radiobiological effects. The massive nuclear fragment known as the alpha particle not only makes direct and glancing collisions with atoms and molecules but also acts as a very efficient ionizer during the "raying" process. Stapleton *et al.* (**1952**) reported that alpha particles are more efficient than X-rays in inactivating *Aspergillus terreus* spores.

Ionizing radiations are injurious to cells that absorb them. Gray (Uber, **1950**) reported that the radiation interfers with almost every known function of the cell when delivered in sufficient dosage. There is an increase in the accumulation of evidence that ionizing radiation destroys enzymes and interferes with the carbohydrate metabolism of the cell. Barron (**1950**) found that ionizing radiation oxidizes the sulfhydryl groups of the cell.

In a previous article (Berk, **1952b**) it was reported that the alpha particle irradiation of *Aspergillus niger* spores in culture medium produced mutants of the fungus. The biological effects

[1] Extract presented before the Society for Industrial Microbiology meeting held in conjunction with the American Institute of Biological Sciences at Cornell University, Ithaca, New York, September 9, 1952.

[2] The author wishes to express his appreciation to the Ordnance Corps of the Department of the Army for permission to publish this article. Special thanks are due to Dr. R. E. Zirkle of the Institute of Radiobiology and Biophysics of the University of Chicago for his helpful comments.

produced were contributed not only by the ionization of the fungus but also by the ionization of the components in the culture medium. In this study dry conidial spores were irradiated with varying doses of alpha particles from polonium and the spores were then germinated on culture medium. An attempt was made to correlate dosage of alpha radiation with inhibition of biological processes in the germination and growth of the fungus.

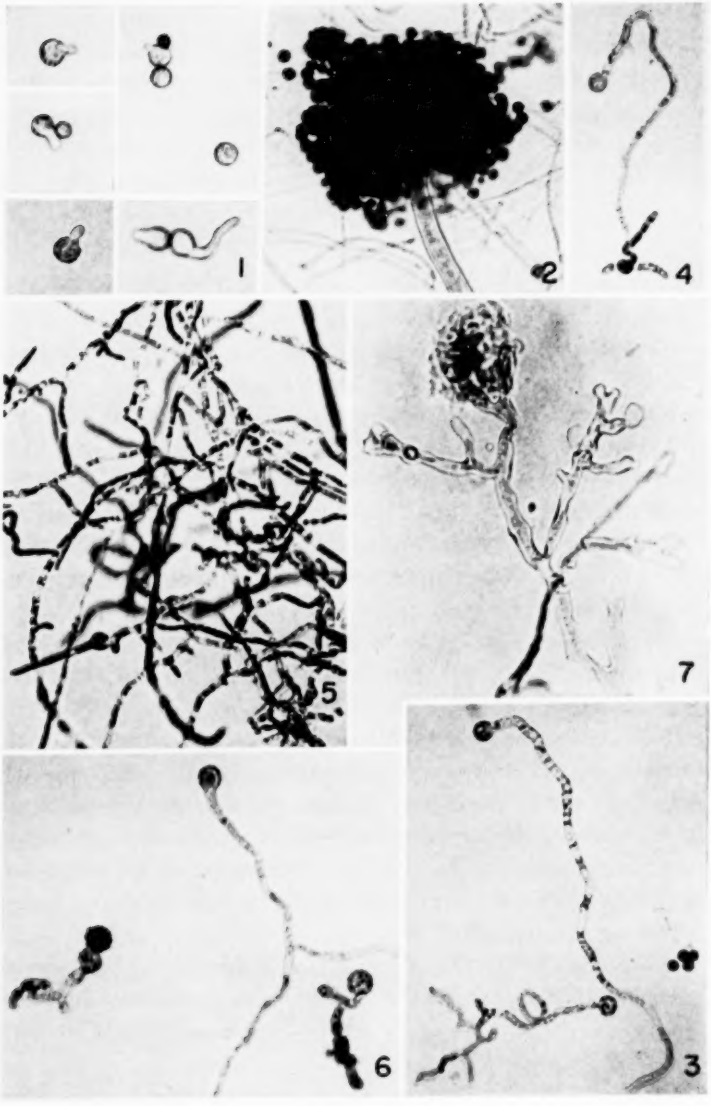
EXPERIMENTAL METHODS

A single spore isolate from a culture of *Aspergillus niger* van Tiegh. USDA TC *215–4247* was used (Berk, **1952b**). This single spore isolate was designated as FA *8300*. Spores harvested from cultures grown on Difco potato-dextrose agar for 3 to 6 days were used to prepare the spore suspensions.

A small drop of the aqueous spore suspension was placed on glass microscope slides which had small circles scratched on the under surface of the slide to facilitate the microscopic examination of the spores. Littman-Well slides were also used (Littman, **1949**). The spore drops were allowed to dry at room temperature and then placed above the radioactive source for irradiation. The slides were then removed from the radioactive source and a drop of melted (40°–45° C) potato-dextrose agar diluted with two parts of distilled water added to the irradiated spores. The slide cultures were incubated over water in 10 cm. desiccators for 17 hours to 6 days in a room maintained at 29° ± 1° C. The 0.5 per cent agar in this culture medium remained in a liquid state at 40° C but solidified immediately upon contact with the microscope slide containing the spores. At the end of the incubation period the thin films of agar formed a flat surface for the mounting medium and cover glass.

After 17 to 144 hours of incubation, a drop of Amann's lactophenol containing cotton blue as a stain was added to the slide. The spores were examined with the compound microscope at a magnification of × 440.

GERMINATION IN ABSENCE OF RADIOACTIVE MATERIALS. After four hours' incubation no visible changes were evident in the spores incubated in the absence of radioactive materials. The



FIGS. 1–7.

spores show the first signs of germination after five hours' incubation by swelling from 5 to 7 microns in diameter. After six hours, the spore swells to 7 to 8 microns and the formation of a germ tube (mycelium beak) is visible. FIG. 1 shows the appearance of the swollen unstained spores after 6½ hours' incubation. After eight hours' incubation most of the spores have the germ tube and show formation of hyphae. From 17 to 24 hours' incubation the germinated spores have entensive mycelial growth (FIG. 5) and show the development of foot cells bearing conidiophore initials. In some of the slides the primary and secondary sterigmata with immature conidia attached were visible.

From 25 to 30 hours' incubation, some of the slides showed a complete conidial apparatus with spores. After 48 hours' incubation all germinated spores had conidiophores bearing chains of mature conidia.

IRRADIATION OF SPORES. The slides containing the dry spores were placed above the polonium sources which were shielded with a stainless steel cutout so that a disc 12.7 mm. (½ in.) in diameter was exposed to the spores. The distance between the slide and the alpha particle source was approximately 13 mm.

The polonium samples consisted of nickel foils plated on one surface with varying amounts of polonium. The active side of the foils was coated with a thin gold film, approximately one micron in thickness, to reduce the contamination hazard. The values used in the experiment are averages of the external radiation of two samples of each concentration used. TABLE 1 lists the calculated activity of the 12.7 mm. diameter discs of polonium at the start and at the finish of the experiments. Eight polonium sources with four activities of polonium varying from 14.7 microcuries (μc.) (5.45×10^5 alpha particles/sec.) to 291 μc. (1.08×10^7 alpha particles/sec.) per disc were used.

The dry spores on the glass slides were irradiated with alpha particles from the polonium sources for lengths of time varying from 20 minutes to 360 hours. Control slides containing spores were exposed in the same 10-inch desiccators that contained the polonium but at a considerable distance beyond the range of the alpha particles.

CALCULATION OF DOSE. The calculation of the dose used is similar to the method described by Zirkle (**1932**).

The total number of alpha particles received by the spores in the experiments ranged from 26 to 74,000. The dose in roentgen-equivalent-physical (rep) received by the spores during these experiments varied from 4800 to 17,000,000.

It is known that the linear energy absorption along the tracks of polonium alpha particles varies in a definite manner (Zirkle, **1940**). Since the range of polonium alpha particles in air is 38.8 mm. and since the range of the alpha particles in tissue having a

TABLE I

INITIAL AND FINAL ACTIVITY OF POLONIUM SOURCES

Source number	Date	Activity in μc. per disc	No. of alpha particles emitted per sec./disc ($\times 10^{-5}$)
1	7/14/49	291	110
1	10/12/49	188	70
2	8/5/49	38	14
2	10/12/49	27	10
3	8/10/49	20	7
3	10/12/49	15	5
4	9/15/49	288	110
4	10/12/49	257	95

density of one is given by Lea (**1947**) as 38.9 μ, 1 mm. of air may be considered equivalent to 1 μ of tissue. The radius of the *Aspergillus niger* spore was taken as 2 μ and the distance from the polonium source to the spore was 13 mm. of air, then the linear energy absorption of the spore in this experiment was approximately 1.3×10^5 electron volts per micron (Zirkle, **1940**). If the distance between the polonium source and the spore had been increased, the linear energy absorption by the spore and also the biological effectiveness of the radiation would have increased. Only a small part of the entire track of the alpha particle is spent in the fungus spore.

BIOLOGICAL EFFECTS ON THE IRRADIATED SPORE

Irradiation of the dry fungus spores with alpha particles produced a number of biological effects when the spores were germinated on the culture medium. In this study observations were concerned primarily with the following inhibitions: Swelling of spore, germ tube formation, vegetative growth, and sporulation.

Zirkle (**1952**) irradiated *Aspergillus terreus* spores with alpha particles. He found that the alpha particle survival curves were exponential when the criterion of survival was the percentage of germ tubes formed from the total number of swollen spores.

When the percentage survival of the irradiated spores was plotted against the dose (expressed either in number of alpha particles or reps absorbed by the spore) on semilog paper, a straight line was not obtained. The nature of the survival curve was such that only approximate values could be stated for the inhibition of the various biological processes. The susceptibility of individual spores to radiation is known to vary. Stapleton and Hollaender (**1952**) reported that there is a definite raltionship between the X-ray sensitivity of spores of *Asperigillus terreus* and their relative water content during the time of irradiation. It is possible that in these studies the water content of the spores may have varied over a wide range since the aqueous drops of spore suspension were air dried at room temperatures with different relative humidities.

TABLE II lists a number of experiments which show the effect of increasing doses of alpha radiation on the percentage of swollen and germinated spores.

The destructive action of the radiation is exerted by the energy absorbed by the spore. It was found that when the spore received less than 100 alpha particles (*23,600* rep) no visible gross effects on germination, vegetative growth, and reproduction of the fungus were noticed.

FIGS. *2* to *28* consist of a number of photomicrographs showing the effect of increasing dosage of alpha particles on the germination, vegetative growth, and reproduction of the fungus. As the number of alpha particles received by the spore is increased from approximately *73* to *8000*, the amount of vegetative mycelial

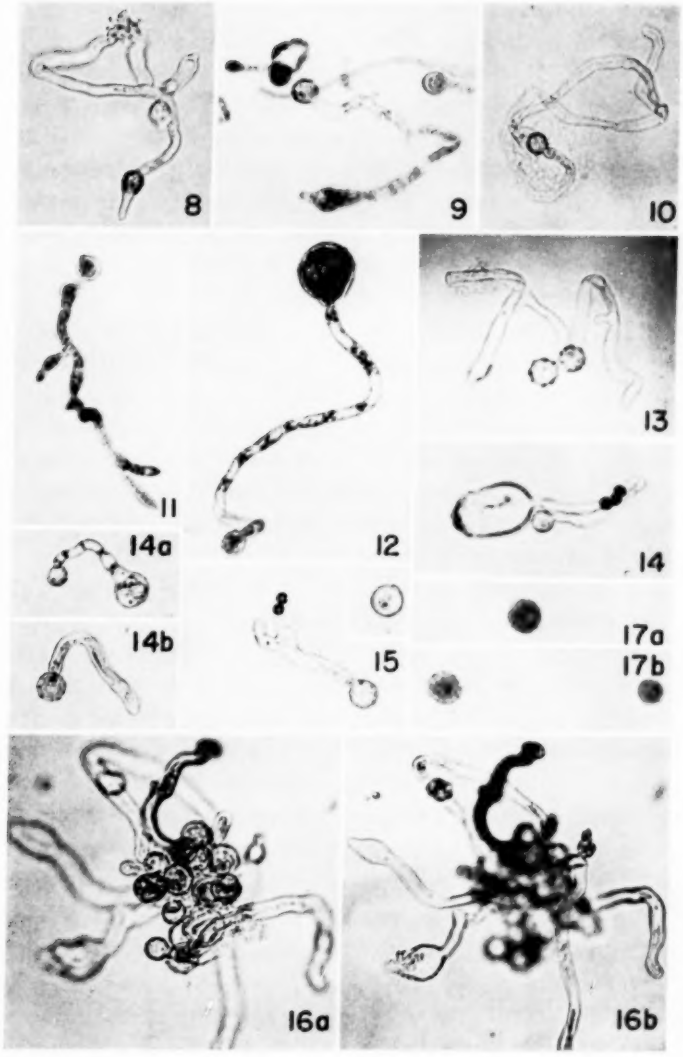
growth is decreased and the number of abnormally germinating spores and lethal mutations is increased. The spores which received 73 alpha particles (13,400 rep) produced spore heads with a complete conidial apparatus after 40 hours' incubation. With low dosages of alpha particles there was such a heavy mycelium growth after 17 to 24 hours' incubation that detailed observations were obscured. FIG. 2 shows that some of the spores which were

TABLE II

EFFECT OF INCREASING DOSAGE OF IONIZING RADIATION FROM POLONIUM ON THE GERMINATION AND SWELLING OF ASPERGILLUS NIGER SPORES
The irradiated spores were incubated on diluted potato-dextrose agar

Polonium source	Dosage, per spore		Incubation of irradiated spores in hours	Per cent spores germinated	Per cent spores swollen	Per cent spores not germinated
	Alpha particles	Rep $\times 10^{-3}$				
1a	140	26	22	92	0	8
1b	200	37	20	72	0	28
3a	360	66	120	69	8	23
4a	460	85	72	63	7	34
3a	550	101	120	57	0	43
2a	630	116	144	47	20	33
3b	1,000	184	96	30	6	64
2a	1,100	202	96	24	10	66
3a	2,000	368	96	29	23	48
2a	2,400	442	20	25	75	0
2a	3,600	662	96	22	29	49
1a	3,900	718	48	12	26	62
1a	4,300	791	50	6	40	54
1a	4,600	846	120	23	53	24
1a	5,800	1,067	72	12	32	56
2b	6,300	1,159	144	11	37	52
1a	7,000	1,288	120	13	50	37
1b	8,100	1,490	96	2	9	89
4a	22,000	4,048	20	12	66	22
1a	26,000	4,784	96	0	2	98
1a	60,000	11,040	96	0	0	100

bombarded with 108 alpha particles (20,000 rep) produced conidial heads with mature conidia that did not differ from the non-irradiated controls. However, all spores that absorbed in excess of 114 alpha particles (21,000 rep) germinated but failed to sporulate. The first visible biological effect of alpha radiation is inhibition of reproduction or sporulation. This effect is often called genostasis.



FIGS. 8–17.

Spores that absorbed 140 alpha particles (26,000 rep) germinated but had restricted mycelial growth (FIG. 3). An example of the profuseness of the mycelial growth of the control is shown in FIG. 5. The control was exposed in the same desiccator as the spores irradiated with 190 alpha particles (35,000 rep) shown in FIG. 4, and both were incubated for 24 hours. As the number of alpha particles which the spore receives is increased, the vegetative growth beyond the germ tube stage is decreased. It appeared that spores receiving in excess of 114 alpha particles lost the ability to produce a living colony but developed abnormally until all growth ceased.

Ford (**1948**) irradiated *Chaetomium globosum* spores with UV and X-rays and then plated the irradiated spores on malt agar. He found that the spores which germinated grew to a certain point and then growth ceased. To these germinated spores he assigned the name "microscopic mutants." A similar effect was obtained from the alpha irradiation of *A. niger* spores. However, a more fitting name would be "lethal mutants." All spores that received in excess of 114 alpha particles and failed to sporulate may be classed as lethal mutants.

The spores that received 290 alpha particles (53,000 rep) and were incubated for 144 hours (FIG. 7) had hyphae with protoplasmic extrusions which are probably produced by the bursting of the swollen hyphae. There was also an increase in the diameter of the hyphae of the irradiated spores over that shown by the non-irradiated controls (compare FIGS. 6, 7, and 18 with FIG. 5).

Owen *et al.* (**1950**) found that when a species of *Botrytis* was treated with nitrogen mustard gas, the germination time of the fungus was delayed. It was thought that possibly the alpha radiation had a delaying effect on the germination and growth of the irradiated spores. Spores which received 320 alpha particles (59,000 rep) to 600 alpha particles (110,000 rep) had limited growth beyond the germ tube stage, even when incubated for 90 hours (FIGS. 8, 10, 11, 15). The effect of alpha irradiation is more than a delayed action. Continued examination of some germinated spores revealed that they had no more than one growth period. The growth of the fungus ceased shortly after the produc-

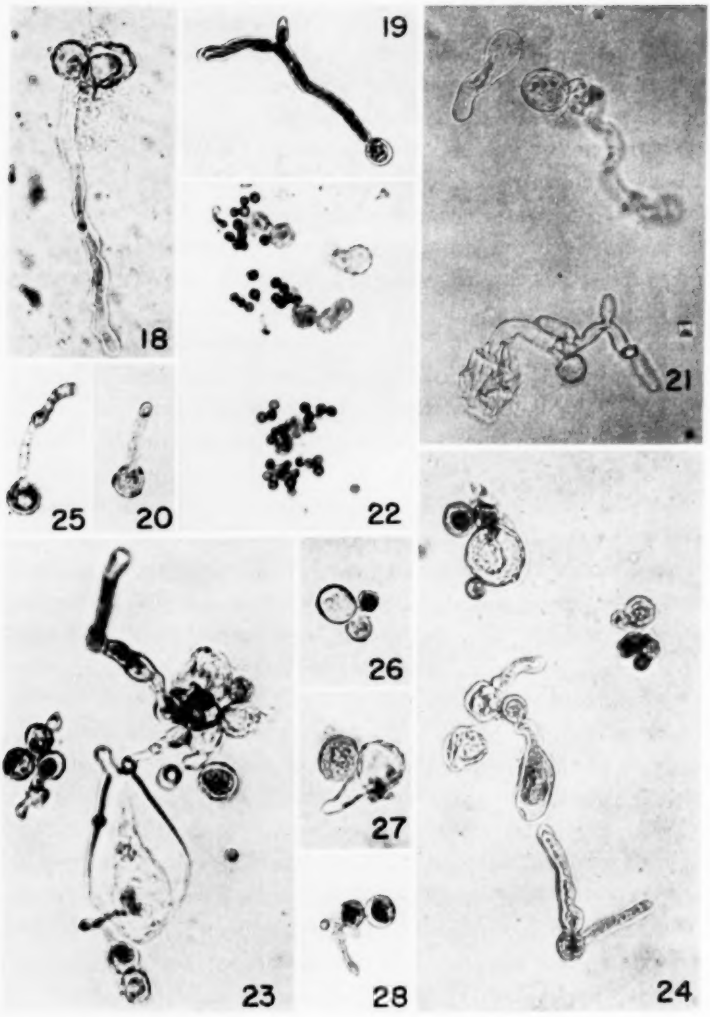
tion of a short, thick germ tube which showed little to no branching.

Dimond and Duggar (**1940**) found that when they incubated spores of *Rhizopus suinus* irradiated with UV, there was no appreciable increase in the amount of growth of the germinated spores from 19 to 30 days' incubation. This raised the question of whether germination constituted a reliable measure of survival. Normally, when a spore germinates the fungus is considered to have survived. In observing the growth of the fungus beyond the germination stage, it was observed that the irradiation had effects on the fungus which did not appear until beyond the germ tube stage. Germination of a spore cannot be considered as a true criterion of the survival of the organism. The true criterion of survival is the ability of the irradiated spore to produce a visible colony with reproductive structures (conidial heads) bearing viable spores.

In FIGS. 9, 12, 14 and 16 the spore received from 360 alpha particles (66,000 rep) to 1170 alpha particles (215,000 rep). The hyphal tips of these germinated spores had swellings, club-shaped and sac-like enlargements, and spherical and pouch-like structures. FIGS. 14 and 14a show spores that have swollen germ tubes which terminate in vesicles. Some of these vesicles disintegrated releasing the protoplasmic contents.

As the number of alpha particles received by the spore is increased (*i.e.*, the higher the dose), the greater the number of restricted growth or lethal mutants produced. Ford and Kirwan (**1949**) found that *Chaetomium globosum* spores irradiated with X-rays produced lethal mutants which varied from 1 per cent for a dose of 9.4×10^3 rep to 100 per cent for a dose of 329×10^3 rep. The alpha particle-irradiated spores germinated but showed little growth beyond the germ tube stage. Many spores had a single straggly hypha; others showed branched and unbranched convoluted hyphae. Growth of the irradiated spores occurs up to a certain point and then ceases (FIGS. 13, 14, 15, 19, 20, 25, 27). These restricted growth mutants may be called lethal mutants since the fungus fails to reproduce a viable colony.

In irradiated spores that received in excess of 1000 alpha particles, the number of swollen spores increases (FIGS. 15, 17a, 17b,



FIGS. 18–28.

22, 23, 24). More than 50 per cent of the spores remained in the swollen condition after 20 hours' incubation (FIG. 17). The spore wall of the giant swollen cell fails to break and the formation of a germ tube is inhibited. With these high doses of radiation, growth

is halted at the giant cell stage (FIGS. 17a, 17b). Butler and Rowell (**1951**) exposed *A. niger* spores to colloidal polonium and also found that the spores became excessively swollen and failed to develop further. In FIG. 21, the spores received 2000 alpha particles and were photographed in the unstained condition after 288 hours' incubation on the agar medium.

The spores in FIG. 22 were suspended in a drop of water and irradiated with 3900 alpha particles while the spores were still wet. The number of ungerminated and swollen spores was very high. One swollen spore appears to have exploded and FIG. 22 shows its extruded protoplasmic contents. Henshaw and Francis (**1935**) reported that spores are more resistant to radiation when dry than when moist. No work beyond this single exposure was done on this problem since the spores dried out on prolonged exposure to the radiation, and the activity of the source was not great enough to permit short exposures of the wet spores. Also the thickness of the drop of water was initially great enough to prevent the passage of the alpha particles to the spore.

Some of the spores that were irradiated with 4950 alpha particles had germ tubes that were much inflated and distended (FIGS. 23, 24). Spores that received 7000 alpha particles (1,288,000 rep) to 8000 alpha particles (1,472,000 rep) had a preponderance of giant swollen cells (FIGS. 26, 27, 28) and did not put out a mycelium beak even when incubated for 72 hours. Some of the swollen spores measured 26 μ in diameter. The swollen cells failed to divide and this inhibition of mitosis is one of the effects of high radiation dosages (Catcheside, **1948**). The few swollen cells that did germinate produced a very short germ tube and then ceased growth (FIGS. 27, 28). Some of the germinated spores had coagulated protoplasm and were probably dead. These spores were probably injured to such an extent by the radiation that they were unable to undergo cell division and perished during mitosis. Nonirradiated spores which were exposed in the same desiccator as the spores shown in FIG. 28 produced normal conidial heads after 48 hours' incubation. Armitage and Verdcourt (**1948**) obtained similar results with sodium pentachlorophenate. With a concentration of 40 to 50 ppm the spores enlarged to giant cells

which did not go beyond the spherical stage for 20 days incubation. Luyet (**1932**) found that when *Rhizopus nigricans* spores were irradiated with cathode rays they swell to giant size without ever producing a mycelium. Ting and Zirkle (**1950**) irradiated blood cells with X-rays and came to the conclusion that the swelling of the cells was due to the increase in the permeability of the cell membrane to dissolved substances.

Complete inhibition of swelling and germination was produced by the irradiation of the spores with 25,000 alpha particles (4,600,000 rep) to 60,000 alpha particles (11,040,000 rep). According to Gottlieb (**1950**) the absorption of water varies with the viability of the spore and he considers a spore dead if it does not swell.

Caution must be exercised in generalizing regarding the biological effects of the alpha particles on the dry spores. Nevertheless, an attempt is made to summarize the results obtained. As the dose of ionizing radiation was increased, there was a progressive decrease in the amount of development shown by the germinating spore until finally with large doses they remained inactive. Spores that received low concentrations of alpha particles (less than 50) showed no visible effects, either inhibitory or stimulatory on germination, vegetative growth, or sporulation. All the spores that received more than 114 alpha particles failed to sporulate (reproduce). Initial inhibition of vegetative development appears in spores bombarded with more than 100 alpha particles. Higher doses of alpha particles (1000 +) produce a large percentage of swollen spores and poor germination. Complete inhibition of swelling and formation of a germ tube results from the irradiation of the spore with more than 25,000 alpha particles. The order in which biological processes in the life cycle of the fungus are interfered with in going from low doses of alpha particles to high doses are: (1) sporulation, (2) vegetative growth or cell division, (3) germination (production of germ tube), and (4) swelling of the spore.

GROWTH OF THE IRRADIATED SPORES ON A COMPLETE MEDIUM. Spores bombarded with more than 114 alpha particles failed to sporulate when incubated on potato-dextrose agar. It was thought that the irradiation might have produced biochemical mutants that

could no longer synthesize the essential amino acids, vitamins, and other essential foods from the potato-dextrose agar used as the culture medium. A modification of the culture medium used by Beadle and Tatum (**1945**) to detect deficiency mutants in *Neurospora* was prepared. A drop of melted medium (45° C) was placed on the nonirradiated and the alpha particle irradiated spores. The nonirradiated control slides had increased vegetative growth and sporulation with the complete medium. No significant improvement in the growth of the irradiated spores was obtained. However, with culture medium containing chemical inhibitors, the effect on the morphology of the fungus is only temporary since normal growth results when the swollen spore is transferred to normal culture medium (Verdcourt, **1952**).

Spores irradiated with 140 to 460 alpha particles and incubated on the complete medium did produce a higher percentage of swollen hyphal tips, pouch-like enlargements at the ends of the hyphae, and protoplasmic extrusions than those incubated on potato-dextrose agar. Ford (**1948**) also found no improvement in the growth of "microscopic mutants" when they were incubated on a more complete medium after irradiation by UV and X-rays. Spores that were irradiated with 32,000 alpha particles failed to swell on the complete medium. This high dose of alpha particles may be considered to be lethal to the spores.

Some of the irradiated spores were also incubated on 3 per cent malt extract. Lindegren (**1949**) and Hollaender *et al.* (**1945**) class malt agar as a more complete medium. In our tests, 3 per cent malt extract failed to stimulate sporulation in the irradiated spores.

DISCUSSION

In the study of the particulate radioactive emanation from polonium on the fungus spore, the dosage was based on the entire spore excluding the spore walls. Hansen and Smith (**1932**) claim that the basic unit of the organism is the nucleus and not the cell (spore). The nucleus has been reported by many as extremely sensitive to radiation. Petrova (**1942**) found the cell nucleus 700 times more radiosensitive to alpha radiation than the cytoplasm. Although Zirkle (**1932, 1949**) reported that nuclear elements were

more susceptible to alpha radiation than the extranuclear ones, he also found that high dosages to the cytoplasm would also be lethal to the cell. Failla (**1937**) reports that ionizing radiation delivers its damage straight to the nucleus through the cell membrane, cytoplasm, and nuclear membrane. At the same time, all the molecules traversed through the irradiated cell become ionized.

The calculation of the number of alpha particles absorbed by the spore nucleus is more difficult since information on the exact dimensions of the nucleus is not available. A number of investigators, Whelden (**1938**), Zahl *et al.* (**1939**), reported that the *Aspergillus niger* nucleus is spherical, and is centrally located in the spore. Wakayama (**1931**) worked on the cytology of the sterigma of the fungus and reported it as uninucleate. The *A. niger* nucleus is described as small and as containing two round small chromosomes in the haploid condition. In the absence of information on the dimensions of the nuclear material of the spore, the size of the sensitive region of the spore was assumed to correspond to the dimensions of the spore minus the thickness of the walls.

In determining the percentage of survival for *A. niger* spores irradiated with the alpha particles some difficulty was experienced in choosing the criteria of survival and death. Zirkle (**1932**) also found that the number of alpha particles required to kill the cell depended on what criteria he used for the death of the cell. More recently Zirkle (**1952**) selected swelling as a criterion of viability of the spore and the protrusion of even a short tube from the swollen spore as a criterion of survival of the fungus. Uber and Goddard (**1934**) reported that the shapes of X-ray survival curves is a function of the criteria selected for death and that the survival curves showed rhythmic variation with dosage. The results obtained in this study revealed that ionizing radiations produce a number of inhibitions in the normal growth processes of the fungus. These inhibitions are: reproduction (sporulation), macroscopically visible mycelial growth, germination (production of germ tube), and swelling of the spore. The dosage of alpha particles required to inhibit these biological processes was found to range from 114 to 25,000. The usual index of death is the inability of the spore to produce a macroscopic colony (Lea, **1946**). Others consider the

failure of a spore to swell as the criterion of death. The author agrees with the criteria of death selected by Uber and Goddard (**1934**) in their work on the effect of X-rays on the fungus *Neurospora*. These criteria are: (1) germination, (2) "positive growth," and (3) presence of normal reproduction and mature conidia. On the basis of these criteria, all spores that received in excess of 100 alpha particles and failed to produce conidia may be considered "dead" or lethal mutants.

The results show that normal growth processes and reproduction are interfered with long before the mechanisms which are responsible for germination and swelling of the fungus spore are destroyed. Very similar biological effects are produced by the irradiation of fungi with alpha particles and X-rays.

The doses required to interfere with cell division were small compared to the lethal dose for the spores. These results are in agreement with the conclusions reached by Spear (1946).

SUMMARY

Dry conidial spores of *Aspergillus niger* were irradiated with varying doses of alpha particles from polonium sources. It was found that the severity of the biological effects is dependent on the dosage or the number of alpha particles directed at the spores.

There is a definite order in which biological processes are interfered with in going from one dose of alpha particles to higher doses. Less than 100 alpha particles (17,400 rep) directed at the spores had no visible stimulatory or inhibitory effects on germination, growth, or sporulation of the fungus. The first visible biological process interfered with is reproduction (sporulation) or production of conidial heads. This inhibition of sporulation occurs when the irradiated spores receive more than 114 alpha particles (21,000 rep). The second process which is inhibited is cell division or the development of an extensive mycelium (vegetative growth). Slight inhibition of vegetative growth begins when the spore receives more than 100 alpha particles. Conidia receiving in excess of 114 alpha particles developed abnormally before all growth processes ceased. As the number of alpha particles received by the spore increases to 1000 (184,000 rep) the extent of

development decreases and the spore loses the ability to develop a living colony. The inhibition of germination or the production of the germ tube is inhibited at about 1000 alpha particles per spore. Above 1000 alpha particles per spore, very few of the irradiated spores develop beyond the swelling stage. A spore that receives more than 25,000 alpha particles (4,600,000 rep) remains inactive.

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EXPLANATION OF FIGURES

FIGS. 1–28. Effect of the number of alpha particles striking the *Aspergillus niger* spore on the germination, growth, and sporulation of the fungus. The irradiated spores were incubated on diluted potato-dextrose agar, stained with Amann's lactophenol plus cotton blue and photographed at a magnification of ×300 except those with an asterisk (*) which are at ×350.

Fig.	*Dosage, per spore*			*Incubation time in hours*
	Polonium source	*Alpha particles*	*Rep.* ×10^{-3}	
1*	Nonirradiated Control[a]			6.5
2	1b	108	20	72
3*	1b	140	26	22
4*	4b	190	35	24
5*	Nonirradiated Control (in same desiccator as 4)			
6*	4b	280	52	20
7*	4a	290	53	144
8	1b	320	59	72
9*	4b	360	66	25
10	1a	398	73	72
11	1b	510	94	40
12	1a	510	94	40
13	3a	550	101	120
14	3b	600	110	90
15	2a	1170	215	43
16	2b	1170	215	43
17*	3a	1300	239	20
18*	2b	1400	258	72
19	2a	1620	298	25
20	2b	1800	331	44
21[b]	3b	2000	368	288
22[b]	1a	3900	718	48
23	1a	4950	911	47
24	1b	4950	911	47
25*	1a	5800	1067	43
26	1a	7000	1288	45
27*	4b	7700	1417	72
28	1a	8000	1472	43

[a] Unstained.
[b] Spores were irradiated in a drop of water.

THE GROWTH AND METABOLIC BEHAVIOR OF STREPTOMYCES VENEZUELAE IN LIQUID CULTURE

DAVID GOTTLIEB[1] AND MARVIN LEGATOR[2]

(WITH 3 FIGURES)

Physiological studies on the growth and metabolism of the actinomycetes have had an interesting history. Very soon after the discovery of these organisms the early investigators probed into the living processes of various species. In 1900, Beijerink (**3**) investigated pigment formation and was followed by Munter (**23**) who studied the nutrition of some members of this group. Krainsky's (**21**) detailed studies in chemically defined media are especially notable since they laid a foundation for many of the investigations which were to come. Through the years that followed, these microbes were almost entirely neglected except for the constant attention of Waksman who published a long series of papers on the metabolism of the actinomycetes (**32, 33, 34, 35**). This hiatus was almost uninterrupted until the advent of antibiotic therapy and the discovery of streptomycin (**28**). An interest in the activities of these organisms was reawakened but the researches were in the main confined to *Streptomyces griseus* (**10, 11, 12, 15, 18, 19, 20, 27, 29, 30, 37**). More recently the studies of Cochrane on *S. coelicolor* have established some of the biochemical systems present in this species (**5, 6, 7, 8, 9**). *S. venezuelae*, the subject of this paper, has also received some attention, but in this instance, too, it has been primarily in relation to antibiotic production (**4, 13, 14, 16, 17, 24, 25, 31**). Further studies of this interesting group of plants are important to determine how their processes fit into the general scheme of other

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living organisms. For this purpose the interrelationship of eight factors in growth and metabolism of *S. venezuelae* were studied; they are the morphological development of the organism, its rate of growth, and the changes in concentration of glycerol, lactic acid, total nitrogen, nitrate nitrogen, ammonia, and hydrogen ion.

METHODS

Streptomyces venezuelae strain 8–44 was used in these experiments. This organism can produce the antibiotic chloramphenicol in some media (**14**, **16**, **17**), but not in the medium used. This medium had the composition given below [3] and was prepared with chemicals of the C.P. or analytical grade, except for the sodium lactate which was available only in U.S.P grade. All observations and analyses were made in triplicate flasks. The medium was dispensed as 100 ml. aliquots in 500 ml. flasks and autoclaved for 20 min. at 15 lbs. pressure. Inoculum of *S. venezuelae* was prepared by growing the organism on modified Emerson agar (**16**) slants. After these cultures were incubated for 7 days at 28° C, 5 ml. of sterile distilled water were added to the slant and the spores rubbed off to make an aqueous suspension. Five ml. of this suspension were added to 100 ml. of liquid medium and the culture was grown for 48 hours on a reciprocal shaker. Two ml. of this culture were used to inoculate each of the flasks used in the experiment. Three flasks, taken at random, were removed from the shaker at various periods of time, from 0 to 192 hours. Aliquots from these flasks were used for all determinations. The morphological studies were made by microscopic observations of both wet mounts and Giemsa stained preparations. Weight of mycelium was obtained by centrifuging the liquid cultures in tared tubes, decanting the supernatant fluid, washing the centrifugate 3 times with distilled water, and finally drying the mycelium at 100° in a vacuum oven for 24 hours. The analytical procedures were adaptations of previously reported methods. Glycerol was

[3] Glycerol—10 gm.; KH_2PO_4—0.7 gm.; $K_2HPO_4 \cdot 3H_2O$—4.0 gm.; Na lactate—11.2 gm.; $NaNO_3$—0.7 gm.; NaCl—3.0 gm.; $MgSO_4$—2.0 gm.; $ZnSO_4 \cdot 7H_2O$—0.0115 gm.; $FeSO_4 \cdot 7H_2O$—0.0111 gm.; $CuSO_4$—0.0064 gm.; $MnCl_2 \cdot 4H_2O$—0.0079 gm.

determined by the colorimetric method of Mikkelson (22) and lactic acid by the method of Barker and Summerson (2). The standard microkjeldahl processes were used for total nitrogen except that the samples were predigested for 16 hours in a steam bath because the samples tended to foam badly during ordinary digestion (1). The analysis for ammonia in the culture fluid was the official method of the A.O.A.C. (1). Nitrate nitrogen was determined by the procedures described by Prince (26). Changes in hydrogen ion concentration of the medium were followed with a glass electrode pH meter.

RESULTS

The growth of *S. venezuelae* began slowly though not with the marked lag which has been described for bacteria (FIG. 2). This phase varied from 12 to 24 hours in the different experiments and was followed for the next 24 to 36 hours by a period of rapid growth which resembled the logarithmic increase of bacterial cultures. Growth rate was then reduced and the total amount of mycelium reached a maximum at about 72 hours. From then on, the total weight slowly decreased and tended to remain constant at about 60 to 70 per cent of the greatest weight. Morphological transformations in the culture corresponded in general to those which have been described for *S. griseus* (18). The spores of *S. venezuelae* germinated within the first 12 hours (FIG. 1 B), and mycelial fragments in the inoculum began visible growth at about the same time. The germ tubes of the spores then elongated into hyphae and side branches formed. This process continued and was accompanied by an anastomosing of the hyphae. At 24 to 36 hours the culture consisted of a large number of wefts of intermingled and anastomosed hyphae, more dense at the center than the edges. New centers of growth also formed from fragments of hyphae which broke off the weft during the agitation. No spores were observed during this period. With increased time of shaking, the size of the mycelial bundles increased; their centers became almost opaque and were surrounded by an open weft which terminated peripherally in young actively growing hyphal tips. Spore formation began at about 48 to 60 hours, and at 72 hours, when the weight of the mycelium was greatest, the culture contained numer-

ous spore chains. In shake culture, no clear differentiation existed between the sporophores and vegetative hyphae of young cultures. When broken from the mycelium the spore chains consisted of from one to many cells.

In these experiments, glycerol was the main source of carbon for the growth of the actinomycete; as the glycerol content of the medium decreased, the mycelium weight increased (FIG. 2). However, the medium was never entirely depleted of this component, and after 72 hours its concentration remained constant. This was the time at which the maximum weight of mycelium occurred and after which lysis began. Under such conditions further utilization of this alcohol would not be reasonably expected.

Lactic acid was not readily dissimilated by *S. venezuelae* although its concentration initially was equal to that of glycerol. Only 20 per cent of the lactic acid was removed from the medium. Growth occurred just as well in the absence as in the presence of this carbon source. That the poor utilization of the lactate was not due to the "sparing effect" of the glycerol was evident from the fact that even as alcohol was gradually depleted the rate of lactate utilization did not increase. No more lactate was consumed when the consumption of glycerol had stopped (FIG. 2). One can assume that the uptake of this component was dependent on the uptake of a more rapidly available carbon source.

The inorganic nitrogen was rapidly removed from the medium during the growth of *S. venezuelae* (FIG. 3). Eighty-six per cent of the nitrate nitrogen disappeared within the first 12 hours and 93 per cent within 48 hours; from then on the concentration of this component remained constant. When total soluble nitrogen was measured, the decrease in concentration in the medium paralleled the decrease of the nitrate ion for the first 60 hours. After 72 hours, the concentration of total soluble nitrogen increased slowly until 144 hours. The drop in total nitrogen in the early phases of growth reflects the removal of the nitrate, while the later increase indicates a lysis of some of the mycelium. This viewpoint is supported by the fact that growth and the total weight of the organism were beginning to decrease or had already ceased during the time of the increase in the total nitrogen concentration. Furthermore,

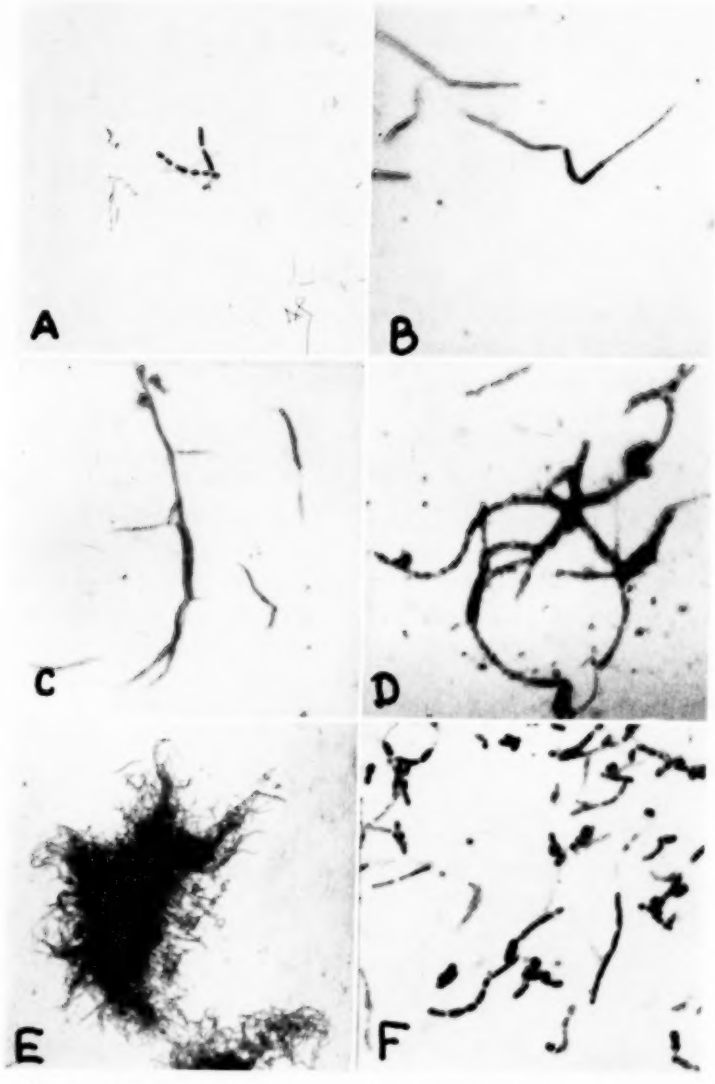

FIG. 1. Morphological changes of *S. venezuelae* in shake culture; A. Spores from inoculum, 12 hr. culture (×970); B. Germinated spores in 12 hr. culture (×970); C. Young hyphae 24 hr. culture (×970); D. Developing mycelium in a 24 hr. culture (×970); E. Mature mycelium in 48 hr. culture (×100); F. Spores in 120 hr. culture (×970).

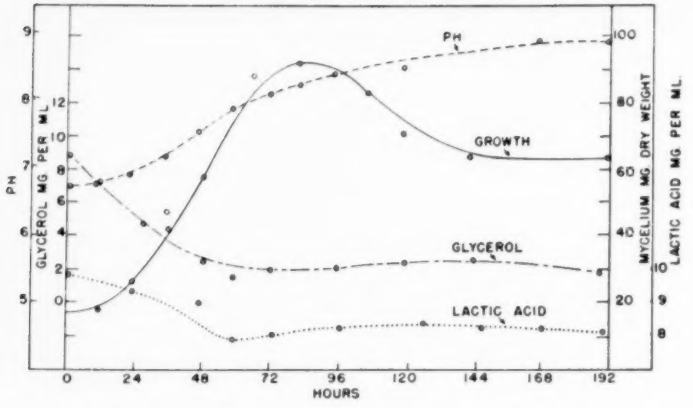

FIG. 2. The relationship between growth of *Streptomyces venezuelae* hydrogen ion concentration, the utilization of glycerol and sodium lactate.

the degradation of the mycelium can be observed microscopically during this period.

Ammonia is apparently a metabolic product during the assimilatory growth period. After a brief lag, which had about the same length as that for growth, the production of this nitrogen com-

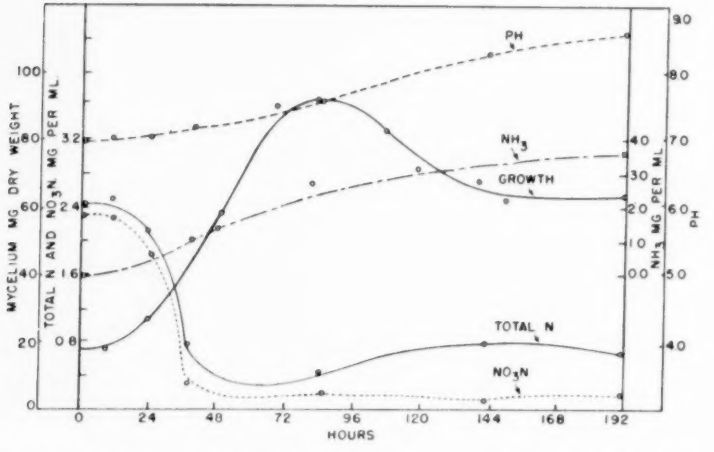

FIG. 3. The changes in various nitrogen components of the medium during the growth of *S. venezuelae*.

pound increased steadily during the first 60 to 72 hours and then the rate of production slowed and finally remained constant.

The hydrogen ion concentration of the medium rose steadily. However, the accumulation of hydroxyl ions can be ascribed to the production of ammonia since the curves for both components are parallel (FIG. 3). With glycerol as the carbon source, free acids are apparently not an important metabolic product.

SUMMARY

The "overall picture" of the growth, the morphological development and the uptake of the various components of a chemically defined medium during the culturing of *Streptomyces venezuelae* is for the most part straightforward. Spores germinate and give rise to hyphae which in turn form a loose mycelial weft. Later the weft changes to a more compact bundle with a dense center surrounded by young growing hyphae. The dry weight of the culture increases during this period; the carbon source, glycerol, disappears at about the time growth ceases. Lactic acid is utilized slightly and only in the period of glycerol utilization. During this time the medium is rapidly depleted of the nitrate ions, and ammonia is released, thereby increasing the hydroxyl ion concentration. Since the nitrate disappears long before the glycerol and before the weight of mycelium reaches its maximum, its assimilation into cellular material must be limited by the relatively low rate of absorption of the carbon source. After growth has reached its maximum, sporulation occurs and the mycelium tends to disintegrate. At this time the total soluble nitrogen in the medium increases because of the lysis of some mycelium.

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THE UTILIZATION OF CARBON AND NITROGEN COMPOUNDS BY USTILAGO ZEAE

FREDERICK T. WOLF

Although *Ustilago zeae* (Beckm.) Unger, the pathogen responsible for the disease known as corn smut, has been cultivated on synthetic media by a number of investigators, information is still incomplete concerning the range of carbon and nitrogen sources which this organism can utilize for growth. In many nutritional studies of fungi, only a single strain has been investigated. The present work is concerned with four monosporidial haploid cultures, representing all the meiotic products obtained from germination of a single chlamydospore. By this means, the role of various substances in the nutrition of several strains of the fungus may be ascertained. Further, possible variations in nutritional characteristics which might originate by meiosis would be revealed.

Maire (**3**) grew *U. zeae* successfully in Raulin's solution, and thus was one of the first workers to cultivate this fungus upon a synthetic medium. Ranker (**5**) studied the growth of nine strains of this organism in 26 different media, in attempts to devise a synthetic medium with optimal capacity for growth. A medium containing K_2SO_4, 0.3 gm.; NH_4NO_3, 0.1 gm.; $CaCl_2$, 0.1 gm.; $Mg_3(PO_4)_2 \cdot 4H_2O$, 0.1 gm.; and glucose 10 gm. per liter was found to be superior to all other combinations tested.

Volkonsky (**8**) studied the utilization of carbohydrates by *U. zeae*, finding that acid was produced from glycerol, xylose, glucose, levulose, galactose, maltose, sucrose, raffinose, and dextrin, but not from arabinose, rhamnose, lactose, or soluble starch. Schopfer and Blumer (**6**), in a study of the vitamin requirements of a number of species of *Ustilago*, established that no growth factors are required by *U. zeae*. Perkins (**4**) confirmed the finding that *U. zeae* was autotrophic with respect to growth factors, and by

ultra-violet irradiation produced a number of mutants heterotrophic for vitamins, amino acids, or nucleic acid components.

MATERIALS AND METHODS

The four monosporidial cultures of *U. zeae* were obtained from Dr. Russell Stevens, University of Tennessee. They were originally isolated with the aid of a micromanipulator, by Dr. Coyt Wilson, Alabama Polytechnic Institute, from the four sporidia of a single promycelium, from a smut gall collected at Auburn, Alabama. The cultures are designated A, B, C and D.

Cultures were grown in 125 cc Erlenmeyer flasks containing 50 cc of nutrient solution. In the experiments in which the carbon source was varied, the basal medium contained $NaNO_3$, 3.0 gm.; KH_2PO_4, 1.0 gm.; $MgSO_4 \cdot 7H_2O$, 0.5 gm.; KCl, 0.5 gm.; and 5 drops of 2% $FeCl_3$ per liter. The carbon sources tested, most of which were obtained from the Pfansteihl Chemical Company, Waukegan, Illinois, were added to the basal medium in quantities providing 400 mg. of carbon per 100 cc, and the pH was adjusted to 6.0.

In the experiments with compounds containing nitrogen, the basal medium contained KH_2PO_4, 1.0 gm.; $MgSO_4 \cdot 7H_2O$, 0.5 gm.; KCl, 0.5 gm.; 5 drops of 2% $FeCl_3$; and sucrose, 30 gm. per liter. The amino acids used were obtained from the Pfansteihl Chemical Company, Waukegan, Illinois; General Biochemicals, Inc., Chagrin Falls, Ohio; and Merck and Company, Rahway, New Jersey. For the inorganic nitrogen sources, glycine, β-alanine, and the *L*-amino acids, the nitrogen level was set at 25 mg. nitrogen per 100 cc. In the case of the *DL*-amino acids, the nitrogen level was increased to 50 mg. nitrogen per 100 cc, on the assumption that the *D*-isomer was unavailable to the fungus. The pH was adjusted to 6.0 in all instances.

Inoculations were made by adding 0.1 cc of a seven day old culture grown in Czapek's solution to each flask, by means of sterile pipettes. Growth was recorded after incubation for ten days at room temperature.

Considerable difficulty was experienced in establishing a satisfactory criterion of growth. This difficulty with *U. zeae* has been

discussed by Ranker (**5**), who found that removal of the cells from the medium by filtration, in an effort to determine weights, was impossibly slow. For this reason Ranker removed the cells by centrifugation, and employed their volume as a growth index. In the present work, filtration was also found to be unsatisfactory. Likewise, turbidity measurements with a Klett-Summerson photoelectric colorimeter were unreliable, because of the mycelial character of certain strains, resulting in the formation of clumps of cells. Growth was therefore estimated subjectively, and recorded on an arbitrary scale of 0, +, ++, +++, and ++++.

RESULTS

Twenty different carbohydrates were tested as sole sources of carbon for growth of each of the four strains of *U. zeae*. The results are presented in TABLE I:

It was found that glucose, levulose, mannose, sucrose, maltose and trehalose were the best of the carbon sources tested, and re-

TABLE I

THE GROWTH OF FOUR STRAINS OF *Ustilago zeae* IN MEDIA CONTAINING VARIOUS SOURCES OF CARBON

Carbon source	Strain			
	A	B	C	C
Glycerol	++	++	++	++
D-Arabinose	0	0	0	0
L-Arabinose	+++	+++	+++	+++
Xylose	+++	+++	+++	+++
Rhamnose	0	0	0	0
Glucose	++++	++++	++++	++++
Levulose	++++	++++	++++	++++
Mannose	++++	++++	++++	++++
Galactose	++	++	++	++
Sorbose	0	0	0	0
Sucrose	++++	++++	++++	++++
Maltose	++++	++++	++++	++++
Trehalose	++++	++++	++++	++++
Lactose	+++	+++	+++	+++
Cellobiose	++	++	++	++
Melibiose	0	0	0	0
Raffinose	+++	+++	+++	+++
Melezitose	+++	+++	+++	+++
Dextrin	+++	+++	+++	+++
Soluble Starch	0	0	0	0
Control (no carbon)	0	0	0	0

TABLE II

THE GROWTH OF FOUR STRAINS OF *Ustilago zeae* IN MEDIA CONTAINING VARIOUS SOURCES OF NITROGEN

Nitrogen source	Strain			
	A	B	C	D
$NaNO_3$	+ + +	+ + +	+ + + +	+ + + +
$(NH_4)_2SO_4$	+ + + +	+ + + +	+ + + +	+ + +
Glycine	+ + +	+ + +	+ +	+ + +
Beta-Alanine	+ + +	+ + +	+ +	+ + +
L-Aspartic Acid	+ + + +	+ + + +	+ + + +	+ + + +
L-Glutamic Acid	+ + +	+ + +	+ + +	+ + +
L-Asparagine	+ + + +	+ + +	+ + +	+ + + +
L-Cystine	0	0	0	0
L-Cysteine.HCl	+	+	+	+
L-Leucine	+ + +	+ + +	+ + +	+ + +
L-Lysine.HCl	+ + +	+ +	+ +	+ +
L-Histidine.HCl	+ +	+ +	+ +	+ +
L-Arginine.HCl	+ + +	+ + +	+ + +	+ + +
L-Tryptophane	+ +	+ +	+ +	+ +
L-Tyrosine	+	+	+	+
L-Proline	+ + +	+ + +	+ + +	+ + +
L-Hydroxyproline	+	+ +	+	+
DL-Alanine	+ + +	+ + +	+ + +	+ +
DL-Serine	+ + + +	+ + + +	+ + + +	+ + + +
DL-Threonine	+ + +	+ + +	+ + +	+ + +
DL-Valine	+ + +	+ + +	+ + +	+ + +
DL-Isoleucine	+ + +	+ + +	+ + +	+ + +
DL-Norleucine	+ + +	+ + +	+ + +	+ + +
DL-Phenylalanine	+ + +	+ + +	+ + +	+ + +
DL-Methionine	+ + +	+ + +	+ +	+ + +
Control (no nitrogen)	0	0	0	0

sulted in maximal amounts of growth. *D*-arabinose, rhamnose, sorbose, melibiose and soluble starch were not utilized. Glycerol, *L*-arabinose, xylose, galactose, lactose, cellobiose, raffinose, melezitose and dextrin were utilized for growth, but the amount of growth produced was not as great as that produced in media containing the hexoses and disaccharides enumerated above. No variation among the four strains in ability to utilize carbohydrates was noted.

Sodium nitrate, ammonium sulphate and 23 different amino acids were compared as sole sources of nitrogen for growth of the four strains of *U. zeae*. The results of this experiment are presented in TABLE II:

Ammonium nitrogen appeared to be as good as nitrate under the conditions of these experiments. Almost all of the amino acids

tested were good sources of nitrogen for *U. zeae*, although no growth occurred in media containing cystine, and very little in media containing cysteine hydrochloride, tyrosine, or hydroxyproline. Aspartic acid, asparagine and serine were the best of the amino acids tested, and gave maximal amounts of growth, comparable to those obtained in ammonium sulphate. Some quantitative differences in amino acid utilization were noted among the four strains tested, but no differences of a qualitative nature were found.

DISCUSSION

When the present findings concerning carbohydrate utilization are compared with those of Volkonsky (**8**) agreement is obtained in all instances except with lactose. It will be recalled that Volkonsky employed acid production rather than growth as a criterion of utilization, which may explain the apparent discrepancy.

De Vay, Rowell and Stakman (**1**) have determined by paper chromatography that extracts of cultures of several strains of *U. zeae* definitely contain ribose, levulose, sucrose and maltose. In addition, arabinose, xylose, rhamnose and cellobiose were less certainly identified. All of these sugars have been shown to be utilized in the present experiments, except for ribose, which was not tested, and rhamnose, which was found not to be utilized. The determination of the sugars present in fungi by means of paper chromatography is deserving of further study in an effort to determine the fate of a single carbohydrate provided to the organism, and to determine the degree of correlation which might be established between results obtained with this technique and those from growth experiments.

Comparison of the present findings may also be made with those of Schopfer and Blumer (**7**) with *U. violacea*, the smut species which has hitherto been most thoroughly studied with respect to its carbon and nitrogen requirements.

The failure of *U. zeae* to attack starch would appear to be of particular interest, in view of the large quantities of starch present in the host plant, and also in view of the good growth obtained in dextrin, maltose, and glucose, which result from starch hydrolysis. The present findings with respect to starch, however, are

confirmatory of those of Gruss (**2**) and Volkonsky (**8**). The good growth on xylose is also of interest because of the large xylan content of the host.

No previous information concerning the utilization of amino acids by *U. zeae* has come to the attention of the writer. De Vay *et al.* (**1**) by paper chromatography have demonstrated the presence of 15 different free amino acids in cells of this species. The present experiments indicate the tryptophane is a rather poor source of nitrogen for growth of *U. zeae*. Tryptophane, however, has been shown to be the precursor of indole acetic acid, which is involved in the pathological changes in plants with corn smut disease (Wolf, **9**).

On the assumption that the utilization of various carbohydrates and amino acids are characters which are inherited and thus are genetically controlled, it might have been expected that segregation would have occurred at meiosis. No qualitative differences in utilization of any of the 20 carbon sources or 25 nitrogen sources were observed, however, among the four haploid strains resulting from meiosis. No information is available concerning the nutritional characteristics of the two strains which presumably mated to produce the chlamydospore from which the four strains studied were derived. It is possible that the original chlamydospore was homozygous for all characters studied. These experiments, therefore, yield little information concerning the mode of inheritance of nutritional characters. The occurrence of quantitative differences in utilization of some of the amino acids among the four strains would suggest the possibility that multiple factors may be involved in the utilization of a single substrate.

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SUMMARY

This study is concerned with the growth of *Ustilago zeae* in synthetic media containing various sources of carbon and nitrogen. Four monosporial cultures of *U. zeae*, all derived from a single promycelium, were studied comparatively. Glucose, levulose,

mannose, sucrose, maltose and trehalose were the best of 20 carbon sources tested. *D*-arabinose, rhamnose, sorbose, melibiose and starch were not utilized. *U. zeae* is able to utilize nitrate, ammonium, or amino nitrogen. Good growth was obtained in 19 of 23 amino acids tested as sole sources of nitrogen, aspartic acid, asparagine, and serine proving to be superior to other amino acids. Little or no growth was obtained with cystine, cysteine hydrochloride, tyrosine, or hydroxyproline. No qualitative differences in utilization of any of these substances were noted among the four strains of *U. zeae* studied.

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COMPARISONS OF THE SURVIVALS OF ASPERGILLUS NIGER SPORES LYOPHILIZED BY VARIOUS METHODS [1]

R. H. Haskins and Joan Anastasiou [2]

(with 1 figure)

Though the fundamentals of freeze-drying were known (but not fully appreciated) at least as early as 1813 (**12**), the first clearly recorded use of sublimation for preserving biological substances was that of Shackell in 1909 (**8**). The preservation of bacteria, viruses, and yeasts by the lyophil process has received considerable attention since then, but only in the last decade have attempts to preserve mold cultures by lyophilization been successful. In this respect the work of Raper and Alexander (**7**) is outstanding.

It has been reported (**11**) that special characteristics may be lost from bacterial cultures during lyophilization. Certain fungi do not seem to be able to survive the lyophil process. Others, while they survive apparently unchanged morphologically, do not retain all of their original characteristics—creating serious problems for the industrial fermentations workers.

It was considered important, therefore, to investigate, methodically, the factors affecting the survival of lyophilized fungous spores, to determine the crucial steps in the various procedures, and to attempt to develop new procedures or to so modify old procedures as to make possible the preservation of all important fungi with retention of all their original characteristics.

[1] Paper presented, in part, before a joint session of the Mycological Society of America and the Microbiological Section of the Botanical Society of America at the Cornell University Meetings of the A.I.B.S., September 8–10, 1952. Issued as Paper No. 153 on the Uses of Plant Products and as N.R.C. No. 3003.

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A number of such studies have been made on bacteria (e.g. Wolff, **11**) while others (e.g. **4**, **5**, **10**) are in progress on fungi. This paper reports the results of several preliminary experiments with the fungus *Aspergillus niger*. The most efficient of the three techniques now in common use for the lyophil preservation of fungous cultures was determined, and modifications of techniques involving pre-lyophilization treatments and various suspending vehicles were examined.

METHODS

Three methods of preservation of fungous spores in the lyophil state were compared in these preliminary studies. The first, for convenience designated the NRRL method, is essentially that of Raper and Alexander (**7**). It involves snap-freezing a suspension of spores in a suitable vehicle, evacuating the tube containing the suspension, first at low temperatures, then eventually at room temperature, and finally sealing off the tubes containing the dried spore suspensions *in vacuo*. During the process the vacuum is not broken.

The second method, for convenience designated the PRL method, is similar to the NRRL method except that the tubes containing the spore suspensions are frozen in a freezer-chest, primary drying is carried out in a vacuum desiccator (**3**) or other suitable container at room temperatures, and secondary drying and sealing off are done on a suitable manifold arrangement. In this process, the vacuum is broken between primary and secondary drying.

The third method is a centrifugal freeze-drying technique, based on work by Greaves (**2**), in which the tubes containing the unfrozen spore suspensions are angle-centrifuged during the initial stages of evacuation to prevent boiling, and until evaporative (auto-) freezing has taken place. After primary drying, the vacuum is broken, and the tubes mounted on manifold where, following secondary drying, they are sealed off, leaving their contents *in vacuo*.

The vacuums used in each of the processes ranged from 100–50 μ mercury pressure during primary drying, and 50–25 μ mercury pressure during secondary drying and sealing off. Tempera-

tures for snap-freeze ranged from -35 to $-65°$ C, and for primary drying according to the NRRL method about $-10°$ C. The vapor trap was maintained at temperatures from -45 to $-65°$ C.

Throughout the work, *Aspergillus niger* PRL *21* was the test organism, and was harvested from the sporulation medium of Gastrock *et al.* (1). The harvesting of spores and the preparation and filling of the tubes were done in the usual manner and procedures standardized as much as possible. Similarly the techniques of opening of the tubes and revival of preserved organisms were standardized and controls included in each test. The percentage survival of spores was determined by direct count of germinated and ungerminated spores following standardized incubation conditions. At least 1000 spores were counted from each lyophil tube tested, with counts from 5–8 tubes included in each average value.

APPARATUS

For the NRRL and PRL techniques and for secondary drying and sealing-off of the centrifuge technique, a small but convenient table apparatus was designed. This apparatus, shown in FIG. 1, consisted of a drying chamber (DC) for primary drying according to the PRL method; a manifold (M) for the whole of the NRRL technique, and the secondary drying and sealing off of both the PRL and centrifuge techniques. A vapor trap (VT) is provided for immersion in a dry-ice, ethylene glycol and water freezing mixture and is so constructed as to be easily and quickly replaced should it become plugged with sublimed water. A large capacity vacuum pump is attached to the central tube (VP) of the vapor trap. A vacuum release (VR) and McLeod vacuum gage (VG) are provided as shown. For more efficient drying the connections between the material to be dried and the vapor trap were made as short and large as practicable.

For the primary drying during the centrifuge technique, an Edwards Model 3PS Centrifuge Freeze-drier[3] was used. This is

[3] Made by W. Edwards & Co. (London) Ltd., Lower Sydenham, London S.E. *26*, England. Canadian distributors: Scientific Exports (Great Britain) Ltd., 50 York Street, Toronto *2*, Canada.

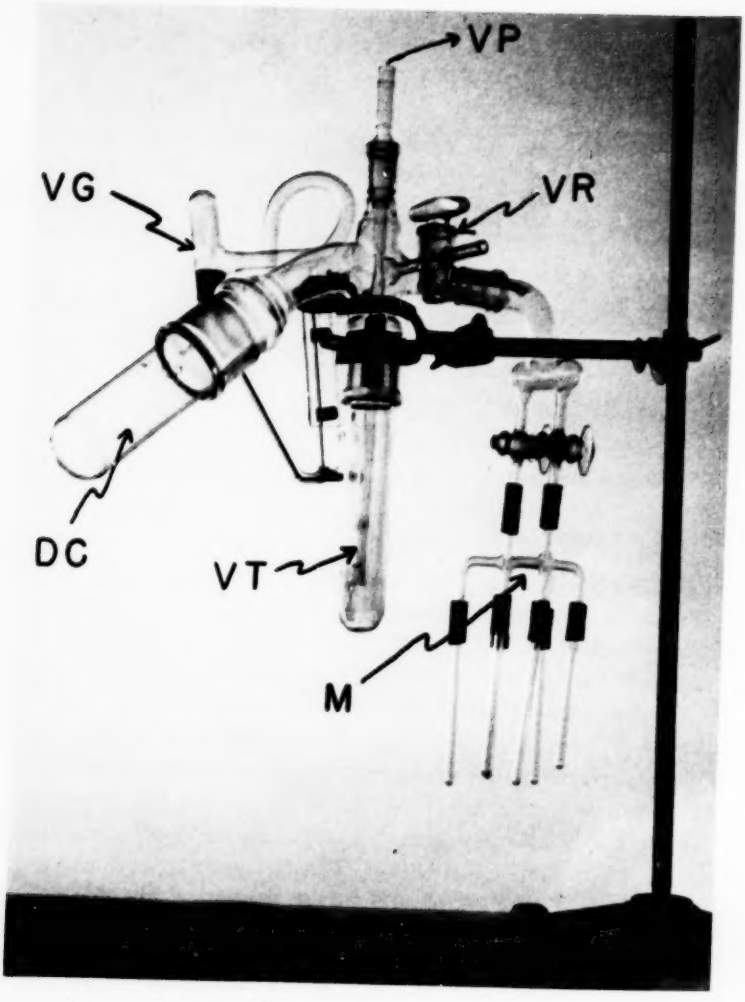

FIG. 1. Table lyophil apparatus. For explanation see text.

a self-contained unit capable of handling, simultaneously, 400 lyophil tubes primary drying, and 400 tubes secondary drying. In our studies, for uniformity, all secondary drying was done on the table apparatus regardless of the lyophil technique being used.

EXPERIMENTAL AND RESULTS

At first, the survival of *A. niger* spores lyophilized in beef serum by the NRRL and by the PRL techniques were compared. The results are shown in TABLE I (a). Controls were treated the same as other tubes except they were not lyophilized, of course, and germination was tested immediately. The table shows that, while the PRL technique gave slightly better results, there was no significant difference in results from the two methods.

Next, both the NRRL and PRL techniques were compared with the centrifuge method. It is obvious from TABLE I (b) and (c) that a significantly greater percentage germination was obtained with the centrifuge technique than with either the NRRL or the PRL techniques.

In 1949 Weston (**10**) reported higher percentage germination of lyophilized fungous spores when the spore suspensions were

TABLE I

COMPARISONS OF THE SURVIVAL OF *A. niger* SPORES LYOPHILIZED IN BEEF SERUM BY VARIOUS TECHNIQUES

Technique	No. of runs	Per cent germination[1]	
		Average	Range
	(a)		
NRRL	7	30.7	10–77
PRL	7	34.1	15–78
Controls	7	93.4	83–98
	(b)		
NRRL	5	32.1	16–58
Centrifuge	5	54.3	44–61
Controls	5	95.4	92–98
	(c)		
PRL	7	53.8	26–75
Centrifuge	7	67.8	47–87
Controls	5	84.4	72–98

[1] NRRL and PRL techniques did not differ significantly. Differences were significant between NRRL and centrifuge techniques ($P < 0.05$) and between PRL and centrifuge techniques ($P < 0.05$).

TABLE II

THE EFFECT OF WETTING AGENTS AND PRE LYOPHILIZATION TREATMENT ON THE SURVIVAL OF *A. niger* SPORES LYOPHILIZED IN BEEF SERUM BY VARIOUS TECHNIQUES

Treatment		Per cent germination[1]					
		NRRL	PRL	Control	Centrifuge		Control
					Average	Range	
without wetting agent	without pre-evacuation	11.8	23.1	91.7	51.3	35–62	
	with pre-evacuation	22.9	45.2		56.9	49–68	86–89
with wetting agent	without pre-evacuation	21.8	20.4	97.2	45.1	30–51	87–88
	with pre-evacuation	34.5	42.1		59.5	46–65	
wetting agent		"Vel"	"Dreft"		"Dreft"		
number of runs		1	1		6		

[1] Analysis of the results for the centrifuge technique only: no significant difference between "with wetting agent" and "without wetting agent" but the difference between "with pre-evacuation" and "without pre-evacuation" was significant ($P < 0.01$).

subjected to dehydration by preliminary evaporation in the liquid state followed by final sublimation from the frozen state. In the next experiment were studied briefly the effects of such pre-lyophilization treatment and of treatment with wetting agents on the survival of *A. niger* spores lyophilized in beef serum by each of the three techniques under consideration. Some of the runs had wetting agent added to the suspending vehicles, others had none added. Both "Vel" and "Dreft" were used as wetting agents. Only sufficient "Vel" or "Dreft" was added to produce a slight foam when the suspension was shaken. This appeared to be enough to wet the spores. In the amounts used, "Vel" and "Dreft" were found to have similarly negligible effects on spore germination. Half of the runs were subjected to reduced pressure at room temperature for 20–30 minutes immediately prior to lyophilization. The pressure was reduced, by means of an aspirator pump, almost to the foaming point of the suspension.

With the NRRL technique (TABLE II) both the use of wetting agents and preliminary evacuation markedly increased per cent germination. With the PRL technique the use of a wetting agent was not beneficial, but preliminary evacuation permitted a 50% increase in germination. With the centrifuge technique, the use of a wetting agent showed no beneficial effect, but preliminary evacuation resulted in significantly increased germination.

This confirms, as first shown by Weston (**10**), that a preliminary evaporation from the liquid state before freezing and drying does result in an increase in per cent germination. This increase does not seem to be due to more efficient wetting of the spore surface, since it was found that letting the spore suspensions stand after preliminary evaporation before lyophilization lessens the effect, indicating perhaps that the beneficial effects of the treatment may be due to a degassing of the spore suspension. There was no such change in the effect of wetting agent when the wetting agents containing suspensions were permitted to stand before processing.

Since the centrifuge technique had proved the most effective of the three methods, it was used in a comparison of various suspending vehicles. The vehicles used and results obtained are shown in TABLE III. Though skim milk has been used extensively as a

TABLE III

A COMPARISON OF THE SURVIVAL OF *A. niger* SPORES LYOPHILIZED IN VARIOUS CARRIERS, USING THE CENTRIFUGE TECHNIQUE

Carrier	Number of runs	Total No. tubes counted	Per cent germination	
			Average	Range
"Kelgin"[1] (0.1%)	3	6	36	15–52
Water	4	9	37	27–56
Beef serum	3	6	43	39–49
Casein hydrolyzate (vitamin free)	4	9	47	15–63
Peptone (10%)	4	6	66	25–76
Sucrose (20%) + ascorbic acid (0.5%)	2	2	71	70–72
Glucose (20%)	2	2	81	78–85
Sucrose (20%) + peptone (10%)	3	3	81	57–99
Sucrose (20%)	6	9	85	70–97

[1] An algin provided by Kelco Company, Los Angeles, California.

suspending vehicle in these laboratories, it could not be included in this comparison, because the opaqueness of material precluded the possibility of determination of per cent germination by direct count.

Of the vehicles reported on here, glucose and sucrose give the best results, confirming the work of Weston (**10**). The use of glucose, sucrose, etc. as vehicles does not result in formation of loose, dried pellets that are so easy to remove from the lyophil tubes when one wishes to revive a lyophilized culture. The use of the centrifuge technique has the same disadvantage, but the increase in per cent germination using such vehicles and such a technique may be sufficient to more than offset this disadvantage, particularly if the methods prove to be more capable of preserving specific physiological characteristics than the methods now in common use.

Though suspending vehicles appear to be emphasized here, their use is not always advisable. Sharp and Smith (**9**) have shown that certain rust uredospores are readily preserved by lyophilization without change in pathogenicity when no suspending vehicle is used, but have no measurable survival when lyophilized in the commonly used suspending media—blood serum, gelatin or sucrose solutions.

DISCUSSION

That fungous spores survive the centrifuge method of freeze-drying better than they survive the other two methods under consideration, is not surprising when the results of studies of the low temperature death of microorganisms (**4**) are considered. With the centrifuge method, while data on the temperature changes in the spore suspension during centrifugation, evacuation and drying are difficult to obtain, it is certain that these changes are in no way as drastic as those imposed during the NRRL and PRL methods.

Wolff (**11**) has shown for bacteria, and Mazur (**4**) for fungi, that the rate of thawing is critical particularly between about -10° C and -35° C. For bacteria, better survival resulted from drying at -35° C than from freezing to -35° C and later raising temperature to -10° C during drying. With fungi, death occurred mostly in rigid frozen vehicles at temperatures between

$-15°$ C and $-35°$ C (**4**), particularly when thawing between these temperatures was slow.

While freezing in the NRRL and PRL methods, as described above, is rapid, raising of the temperatures of the frozen suspensions is carried out more slowly, perhaps accounting in part for the lower survival permitted by these methods.

Why a preliminary evaporation from the liquid state followed by sublimation from the frozen state should give a survival better than when the preliminary evaporation is omitted, is not yet understood. It does not appear to be a wetting phenomenon. Studies on the degassing effect of the treatment are continuing.

The problem of which suspending vehicle to use assumed considerable importance when it was discovered (**10**) that 20% sucrose gave much better survival with fungous spores than did the "protective colloids" so emphasized by workers studying preservation of bacteria (e.g. **6**), and when it was shown (**9**) that the use of suspending vehicles is not always advisable.

It is probable that no single technique will be developed that will be equally suitable for the preservation of all microorganisms. The particular property or properties of the organism upon which selection of the lyophilization technique hinges have yet to be discovered.

SUMMARY

1. The percentage germination of *Aspergillus niger* spores following a centrifuge freeze-drying method was significantly higher than that following two other commonly used methods involving snap-freezing to low temperatures.

2. The higher per cent germination resulting from a preliminary evaporation in the liquid state followed by final sublimation from the frozen state did not appear to be due to more efficient wetting of the spore surface, but rather to a degassing effect.

3. Of the limited series of suspending vehicles tested with the centrifuge method, 20% glucose or sucrose permitted the greatest survival (over 80%) of the fungous spores.

4. The use of such vehicles and of the centrifuge method of preserving spores does not result in the formation of loose dried pellets which are easily removed from the tube when the culture

is to be revived, but the increase in survival may be sufficient to more than offset this disadvantage.

ACKNOWLEDGMENTS

The authors are greatly indebted to Mr. Wm. Hogg for construction of the table apparatus shown in the figure, to Dr. D. Rudd Jones for statistical analyses, and to Drs. Jones and A. C. Blackwood for criticisms of the manuscript.

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THE PRODUCTION AND ACTION OF A TARTRATE-DECOMPOSING ENZYME

RICHARD R. BARTON

(WITH 4 FIGURES)

At the present time, the principal method of eliminating argols (crude potassium bitartrate crystals) from grape products is to speed their crystallization and to remove the crystals by filtration. Despite the fact that many micro-organisms are characterized by their ability to utilize tartrates (Fulmer-Werkman, **5**; Stadtman, Vaughn and Marsh, **14**), there has been no effort to use them or their enzyme systems to prevent argol formation.

Several fungi were isolated by Powers (**11**) from wine lees and grape pomace piles that were capable of utilizing potassium bitartrate as their sole source of carbon. Of these, a strain of *Aspergillus versicolor* (Vuill.) Tiraboschi was the most effective in that it utilized 87.7 per cent of the tartrates in solution. In order to utilize this fungus for tartrase production, it was necessary to ascertain the cultural method best suited for enzyme production, to determine the ability of various strains of this fungus to produce an active enzyme, to determine the relation between metallic salts added to the substrate and enzyme activity, to ascertain the relationship between external factors and tartrase activity, and to identify the break-down product of the enzyme catalyzed reaction.

METHODS AND MATERIALS

Culture of Aspergillus versicolor and extraction of tartrase. Investigation showed that the medium most suitable for the culture of *A. versicolor* for tartrase production was ground grape pomace containing an added 1 per cent potassium bitratrate with the pH adjusted to 3.0. Fifty gram portions of this medium were spread on the bottom of 500 ml. wide mouth Erlenmeyer flasks and sterilized at 15 pounds pressure for 20 minutes. In order to obtain

rapid fungus growth on this medium, it was necessary to first introduce the fungus spores in a liquid medium containing 0.7 per cent yeast extract, 0.5 per cent potassium acid phosphate and 5 per cent dextrose in order to obtain germination. About 10 ml. of this medium containing the germinated spores were used to inoculate 50 grams of the solid medium. Heavy mycelial growth was obtained in three to seven days incubation at 30° C, depending on the strain of the fungus used.

For maximum tartrase activity, the enzyme was extracted from the fungus just before spore formation occurred. Fifty grams of the moldy medium were macerated and mixed with 500 ml. of cold (8° C) water and stirred for one hour to extract the tartrase. In order to remove particles of medium and mycelia, about 200 grams of filter aid were mixed with the extract before filtration through a Buchner funnel.

As with several other enzymes (Crewther and Lennox, **2**; Stark and Tetrault, **15**; and Tauber, **16**), tartrase was insoluble in ethyl alcohol. In order to precipitate the enzyme from the extract, two volumes of cold (8° C) 95 per cent ethyl alcohol were added to each volume of enzyme extract. The enzyme was separated from the supernatant liquid by means of a centrifuge. This moist enzyme preparation was used to ascertain the tartrase activity of the different strains of *A. versicolor* tested and to study the relation between metallic salts added to the substrate and tartrase activity. Since this moist preparation would lose its activity within 24 hours, it was necessary to dry it in order to obtain a stable preparation. Using an adaption of the apparatus of Flosdorf (**3**), the enzyme was dried by lyophilization.

Ability of different strains of Aspergillus versicolor to produce an active enzyme. The ability of different strains of fungi to utilize certain nutrient substances or to produce active enzymes varies over a wide range (Foster, **4**; Harter and Weimer, **7**; Menon, **10**; and Raper, Alexander and Coghill, **12**). Eight different strains of *A. versicolor* were obtained from different sources. From the strain which produced the most active tartrase, 91 single spore isolate cultures were obtained by means of a micromanipulator. In addition, several of these single spore isolate

cultures were treated with radio-active phosphorus in an effort to obtain mutants which would produce more active enzyme preparations.

To use a uniform amount of material from each culture, the enzyme from 50 ml. of aqueous extract was added to 100 ml. of a 1 per cent tartaric acid or potassium bitartrate substrate. After incubation of 30° C for 20 hours, the tartrase activity was ascertained by determining the per cent tartrate decomposition using the sodium metavanadate colorimetric determination of Underhill, Peterman and Kraus (**17**).

Since it was found that tartrase was an adaptive enzyme (Virtanen, **18**), it was necessary to culture the different strains of *A. versicolor* on slants that contained potassium bitartrate as a major source of carbon in order to retain their original tartrase activity.

Relationship between metallic salts added to the substrate and tartrase activity. This work was divided into two sections. In the first part the following concentrations of metallic salts were used: ferrous chloride, 0.1 per cent; zinc chloride, 0.1 per cent; cuprous chloride, 0.0005 per cent; ammonium molybdate, 0.01 per cent; magnesium sulfate, 0.25 per cent, and manganese sulfate, 0.02 per cent. Further work was done using a combination of ferrous chloride and other salts under test. The second part of this work was to determine the action of various concentrations of ferrous chloride and a combination of ferrous chloride and zinc sulfate. The ferrous chloride was used in concentrations ranging from 0.04 to 0.20 per cent in 0.02 per cent increments. The concentrations of ferrous chloride and zinc sulfate used were the same. In each case, however, the total salt concentration was maintained at 0.24 per cent.

The enzyme from 50 ml. of enzyme extract was added to 100 ml. of substrate containing the added metallic salts. After 20 hours incubation at 30° C, enzyme activity was determined. Three replicates of each determination were made.

Relation between external factors and tartrase activity. To ascertain the relationship between time, temperature and pH of the substrate and tartrase activity, 10 mg. of a dried tartrase prepara-

tion and 8 mg. of ferrous chloride were added to 10 ml. of a 1 per cent potassium bitartrate substrate. The same amounts of tartrase and ferrous chloride were added to 10 ml. aliquots of substrate containing varying concentrations of potassium bitartrate to determine the relationship between concentration of substrate and tartrase activity. Concentrations of tartrase ranging from 0.005 to 1.00 per cent were used in the study of the relationship between tartrase activity and concentration.

Identification of enzymatic breakdown products of tartaric acid. Total acid and pH measurements of the substrate before and after the enzymatic reaction had occurred indicated that there was very little change in either measurement and that there was no trend towards either greater acidity or alkalinity. Because of this it was assumed that the breakdown product was an acid. The filter paper chromatogram procedure of Lugg and Overell (**9**), was used to identify this acid.

RESULTS

Ability of strains of Aspergillus versicolor to produce tartrase. The eight strains of *A. versicolor* obtained from different sources produced tartrase preparations of varying activity (TABLE I). This activity ranged from 36.1 to 49.1 per cent tartrate decomposition when the tartrates were supplied in the form of tartaric acid.

The tartrase from 30 of the 91 single spore isolates obtained from the culture of *A. versicolor* obtained from Dr. W. D. Gray

TABLE I

ENZYMATIC DECOMPOSITION OF TARTARIC ACID BY ENZYMES EXTRACTED FROM STRAINS OF *Aspergillus versicolor*

Source and strain number	Initial tartrate concentration, mg./ml.	Final tartrate concentration, mg./ml.	Per cent decomposition[1]
O.A.E.S. *74*	10.8	6.8	38.1
Gray (OSU) *67*	10.8	5.5	49.1
NRRL *226*	10.5	5.4	48.5
NRRL *227*	10.8	6.5	39.8
NRRL *231*	10.8	6.5	39.8
NRRL *233*	11.0	6.5	40.9
NRRL *235*	10.5	5.4	48.5
NRRL *238*	10.8	6.9	36.1

[1] Incubation time, 20 hours at 30° C.

TABLE II

TARTRASE ACTIVITY OF ENZYMES EXTRACTED FROM STRAINS OF *Aspergillus versicolor* GROWN ON MEDIA CONTAINING RADIO ACTIVE PHOSPHORUS

Strain No.	Before irradiation	Tartrase activity[1]	
		Immediately after subculturing from irradiation medium	Six months after treatment
4	22.9	37.6	22.9
8	19.3	39.3	18.3
9	22.9	32.8	22.5
10	25.3	39.3	25.7
11	22.9	32.8	23.0
13	20.4	45.0	20.6
14	25.3	37.6	25.7
15	19.3	41.8	19.5

[1] Expressed as percent tartrate decomposition.

of the Botany Department of The Ohio State University was tested on a tartaric acid substrate. It was found that the activity of these preparations ranged from a low of 30.6 per cent decomposition to a high of 64.5 per cent decomposition. On analysis it was found that the mean decomposition was 46.6 per cent and the standard deviation was 9.26 per cent.

Since the argols in grape products consist largely of potassium bitartrate, the tartrase from 81 of the 91 single spore isolate cultures was tested on substrates containing this salt. Tartrase activity was considerably lower against potassium bitartrate than against tartaric acid, ranging from 1.3 to 34.2 per cent decomposition. The mean decomposition was 22.3 per cent and the standard deviation was 6.05 per cent.

From eight of the single spore isolate cultures treated with radioactive phosphorus, the tartrase was extracted and tested immediately after treatment and again six months later. As can be seen in TABLE II, immediately after treatment with this isotope, the enzymes were more active (an average of 16 per cent higher) but six months after treatment their activities had dropped back to what it was in the original single spore isolates.

Single spore isolate culture No. 76 produced the most active tartrase and was used as the source of this enzyme for the remainder of the work.

TABLE III

RELATION BETWEEN METALLIC SALTS ADDED TO SUBSTRATE AND TARTRASE ACTIVITY[1]

Salt	Concentration		Per cent potassium bitartrate decomposition[2]
	Gms./100 ml.	Molarity	
$ZnSO_4$	0.100	0.0062	29.0
$FeCl_2$	0.100	0.0079	37.5
$CuCl_2$	0.0005	0.000037	26.3
$(NH_4)_2MoO_4$	0.001	0.000055	22.5
$MgSO_4$	0.250	0.0206	23.5
$MnSO_4$	0.020	0.0013	20.5
Control	—	—	15.5

[1] Single spore culture No. *76* used.
[2] Average of three replications.
LSD 3.21 and 4.45 at 5% and 1% levels.

Activation of tartrase by addition of metallic salts to the substrate. When iron, zinc and copper salts were added to the substrate, tartrase activity was significantly higher at the 1 per cent level than that of the control (22.0 to 10.8 per cent higher). When molybdenum, magnesium and manganese salts were present enzyme activity was significantly higher at the 5 per cent level (TABLE III).

In the presence of a combination of iron and the other salts tartrase activity was higher in the presence of a combination of

TABLE IV

RELATION BETWEEN COMBINATION OF IRON AND OTHER METALLIC SALTS ADDED TO SUBSTRATE AND TARTRASE ACTIVITY[1]

$FeCl_2$ Gms./100 ml.	Molarity	Metallic salt	Concentration		Per cent potassium bitartrate decomposition[2]
			Gms./100 ml.	Molarity	
—	—	$FeSO_4$	0.100	0.0066	30.7
0.100	.0079	—	—	—	32.1
0.100	.0079	$ZnSO_4$	0.100	0.0062	36.8
0.100	.0079	$CuCl_2$	0.0005	0.000037	36.2
0.100	.0079	$(NH_4)_2MoO_4$	0.001	0.000055	33.6
0.100	.0079	$MgSO_4$	0.250	0.0206	39.5
0.100	.0079	$MnSO_4$	0.020	0.0013	37.5
Control		—	—	—	29.7

[1] Single spore culture No. *76* used.
[2] Average of three replications.
LSD 2.86 and 3.97 at 5% and 1% levels.

TABLE V

RELATION BETWEEN VARYING CONCENTRATIONS OF $FeCl_2$ AND $ZnSO_4$ ADDED TO SUBSTRATE AND TARTRASE ACTIVITY[1]

Salt concentration, gms./100 ml.		Per cent potassium bitartrate decomposition[2]
$FeCl_2$	$ZnSO_4$	
0.04	0.20	22.2
0.06	0.18	24.1
0.08	0.16	28.1
0.10	0.14	29.2
0.12	0.12	26.0
0.14	0.10	31.1
0.16	0.08	31.1
0.18	0.06	31.5
0.20	0.04	33.0
Control	—	18.1

[1] Single spore culture No. *76* used.
[2] Average of three replications.
LSD 1.45 and 1.99 at the 5% and 1% levels.

iron and zinc, iron and copper, iron and magnesium, and iron and manganese than in the presence of iron alone (TABLE IV).

When varying concentrations of iron and zinc were added to the substrate, tartrase activity increased from 22.2 per cent decomposition when 0.04 per cent iron and 0.20 per cent zinc were used to 33.0 per cent decomposition when 0.20 per cent iron and 0.04 per cent zinc were present (TABLE V). In the presence of varying iron concentrations, tartrase activity increased from 22.6 per

TABLE VI

RELATION BETWEEN VARYING CONCENTRATIONS OF $FeCl_2$ ADDED TO SUBSTRATE AND TARTRASE ACTIVITY[1]

Concentration of $FeCl_2$ gms./100 ml.	Per cent potassium bitartrate decomposition[2]
0.04	22.6
0.06	23.5
0.08	26.6
0.10	28.5
0.12	27.3
0.14	30.1
0.16	28.5
0.18	31.6
0.20	31.6
Control	21.5

[1] Single spore culture No. *76* used.
[2] Average of three replications.
LSD 3.08 and 4.15 at 5% and 1% levels.

cent decomposition to 31.6 per cent decomposition as the iron concentration increased from 0.04 to 0.20 per cent (TABLE VI).

Relation between external factors and tartrase activity. In this work, in so far as possible, all factors were kept constant except the one under study.

Tartrase activity was determined at time intervals of 2, 4, 6, 8 and 26 hours incubation at 40° C. The potassium bitartrate concentrations at the beginning and the end of each time interval were

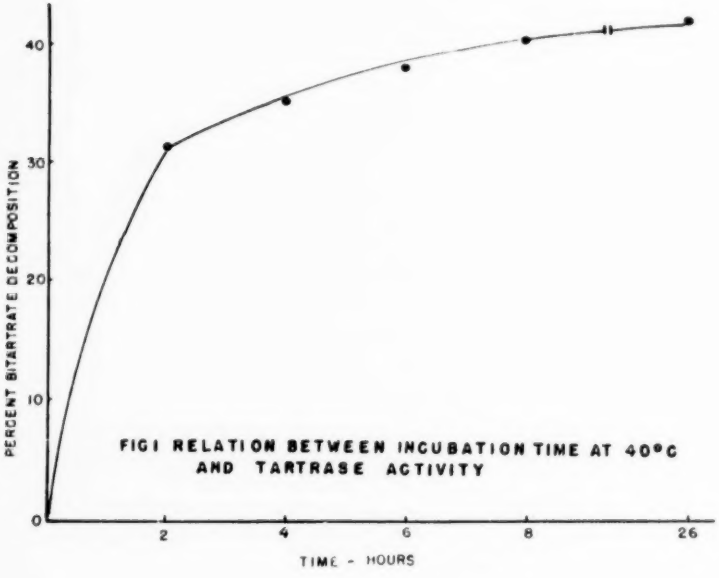

FIG. 1. RELATION BETWEEN INCUBATION TIME AT 40°C AND TARTRASE ACTIVITY

calculated as molar concentrations in order to compute the velocity constants of the enzyme catalyzed reaction. These constants were calculated using the first order reaction equation. As can be seen in FIG. 1, 78.8 per cent of the total enzyme activity occurred in the first two hours of incubation and then increased slowly. The velocity constants decreased from a high of 0.0882 at two hours incubation to a low of 0.008 at 26 hours incubation. It can also be seen that at the end of eight hours incubation 96.0 per cent of the total tartrate decomposition had occurred.

In this study substrate-enzyme mixtures were incubated for two hours at 20° C, 30° C, 40° C, 50° C, and 60° C. From FIG. 2 it can be seen that the optimum temperature for this enzyme catalyzed reaction was 50° C. The velocity constants which were calculated with the same equation used above increased as the temperature increased until a maximum of 0.0994 was reached at 50° C and then decreased as the temperature increased further. At 50° C the tartrase activity was 36.8 per cent decomposition which was significantly higher than at 40° C or 60° C.

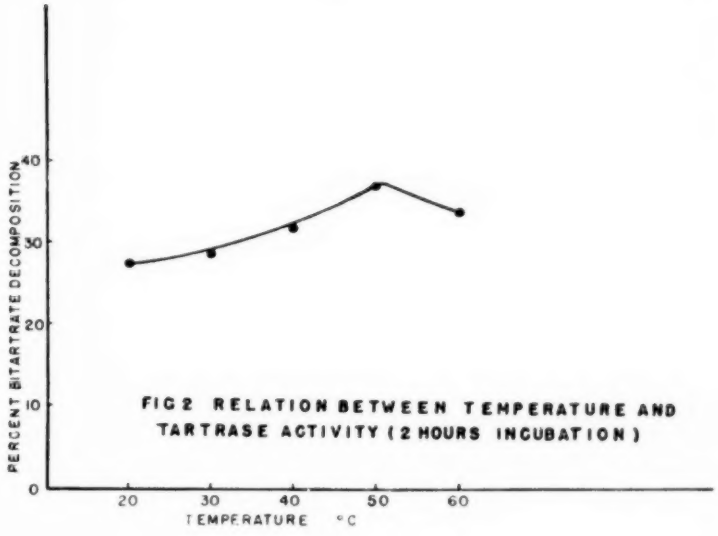

FIG 2 RELATION BETWEEN TEMPERATURE AND TARTRASE ACTIVITY (2 HOURS INCUBATION)

Mixtures of substrates with initial pH of 2.0, 3.0, 4.0, 5.0 and 6.0 and the enzyme were incubated at 30° C for four hours. As can be seen in FIG. 3, the per cent decomposition decreased significantly from a high of 45.0 per cent at pH 2.0 to a low of 23.8 at pH 6.0.

The substrates containing 0.25, 0.50, 1.0 and 2.0 per cent potassium bitartrate were incubated at 30° C for four hours. Enzymatic activity was calculated as per cent decomposition and as milligrams of tartrate per milliliter decomposition. In FIG. 4, it can be seen that per cent decomposition decreased from a high of

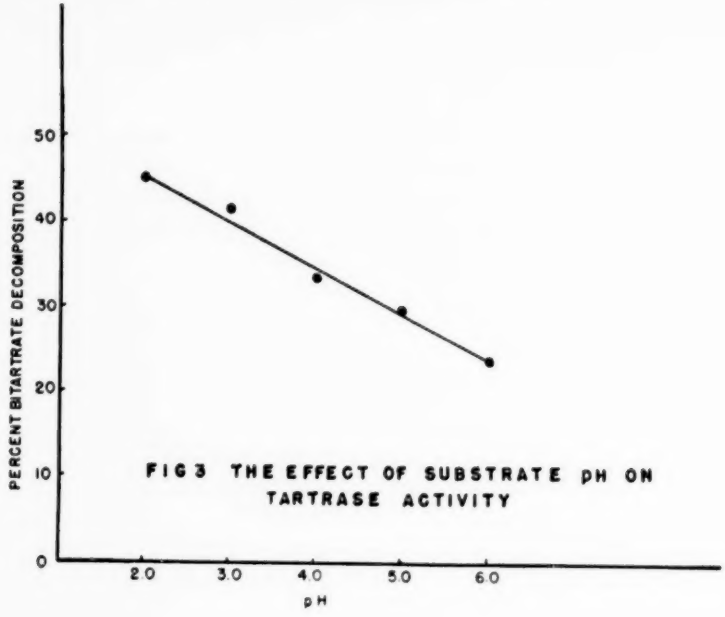

FIG 3 THE EFFECT OF SUBSTRATE pH ON TARTRASE ACTIVITY

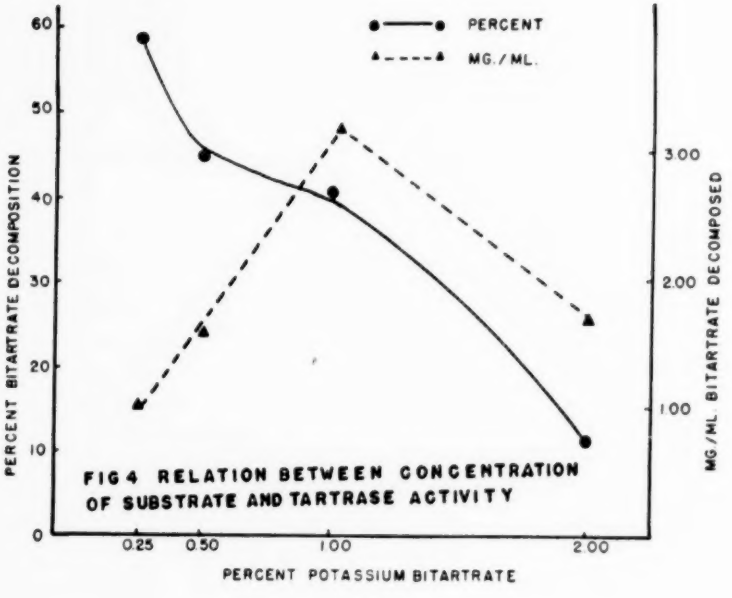

FIG 4 RELATION BETWEEN CONCENTRATION OF SUBSTRATE AND TARTRASE ACTIVITY

58.4 per cent at a substrate concentration of 0.25 per cent to a low of 11.1 per cent decomposition at a substrate concentration of 2.0 per cent. When activity was calculated as mg./ml. decomposition, it was found that activity increased from 1.05 mg./ml. at the lowest concentration to a high of 3.20 mg./ml. at a substrate concentration of 1.0 per cent and then decreased as the concentration of the substrate continued to increase.

This study was made on several different determinations as enzyme concentrations from 0.005 to 1.0 per cent were used to ascertain whether enzyme concentration influenced the rate of reaction. It was found that tartrase activity was significantly higher when used

TABLE VII

RELATION BETWEEN CONCENTRATION OF ENZYME AND TARTRASE ACTIVITY[1]

Enzyme concentration, per cent	Initial tartrate concentration, mg./ml.	Final tartrate concentration, mg./ml.	Per cent tartrate decomposition[2]
0.005	8.8	6.6	29.6
0.010	8.2	5.5	32.9
0.050	8.2	5.6	31.7
0.100	8.2	5.6	31.7
0.150	8.2	5.6	31.7
0.200	8.0	5.2	34.9
0.500	8.8	5.6	32.9
1.000	8.8	6.3	28.4

[1] As determined after incubation at 30° C for 20 hours.
[2] Average of three replications.
LSD 1.84 and 2.55 at 5% and 1% levels.

in concentrations ranging from 0.01 to 0.50 per cent than when used at higher and lower concentrations. The highest activity (34.9 per cent decomposition) occurred when the enzyme was used in a concentration of 0.20 per cent (TABLE VII).

Identification of enzymatic breakdown product of tartaric acid. After the chromatograms were developed, the substrate unknowns had separated into two definite spots. As can be seen in TABLE VIII, the Rf values of these spots checked very closely with those of tartaric and succinic acids.

Action of tartrase in Concord grape juice. When tartrase decomposed more than 30.0 per cent of the tartrates in a pure solution, there was no formation of potassium bitartrate crystals.

TABLE VIII

FILTER PAPER CHROMATOGRAM RESULTS OF IDENTIFICATION OF ENZYME BREAKDOWN PRODUCTS OF TARTARIC ACID[1]

Acid	Rf value		
	1	2	3
Succinic	0.804	0.760	0.759
Fumaric	0.886	0.852	0.875
Tartaric	0.189	0.188	0.175
Unknown No. 1	0.196	0.180	0.171
Unknown No. 2	0.800	0.768	0.761

[1] Determination made on tartaric acid substrate after 20 hours incubation at 40° C.

However, enzyme activity was erratic in decomposing the tartrates present in Concord grape juice. When tested on grape juice of the 1948 season, tartrate decomposition ranged from 24.5 to 38.2 per cent. In tests made on 1951 season juice, tartrase activity ranged from 0 to 21.2 per cent decomposition. These low activities were due to the presence of an inhibitor. Mixing small quantities of ion-exchange resins with the tartrase before addition to the juice only partially removed the inhibitor.

DISCUSSION

It was shown that a strain of *A. versicolor* could produce a tartrate decomposing enzyme sufficiently active to prevent the formation of potassium bitartrate crystals in a solution containing this salt in approximately the concentration that it is found in grape juice. That the form in which the tartrates are present influences the activity of tartrase can be seen in that tartaric acid was decomposed to a greater extent than is potassium bitartrate. As in the case of other fungal enzymes (Foster, **4**; Harter & Weimer, **7**), tartrase activity varies with the strain of the organism producing it. Tartrase is an adaptive enzyme (Virtanen, **18**), as indicated by the fact that its activity decreases if the fungus from which it is extracted is not cultured on a medium containing potassium bitartrate as a major source of carbon.

The activation of tartrase by metallic salts is in agreement with the work of Linderstrom-Lang (**18**); Baumann and Heumuller

(**1**); and Smith and Bergman (**13**) on the activation of enzymes by metallic salts.

The effect of time, temperature and concentration of substrate on tartrase activity is similar to their effects on other enzymes (Gortner, **6**). While there is probably an optimum pH for tartrase action, it cannot be shown using potassium bitartrate as a substrate. This is due to the fact that as the pH decreases, increasing amounts of this salt are converted to tartaric acid. As has been shown above, tartrase is more effective in decomposing tartaric acid than potassium bitartrate. While the concentration of tartrase influences enzyme activity, this influence is not proportional to the enzyme concentration as reported by Gortner (**6**).

Despite the fact that tartrase preparations have been obtained that prevent the formation of potassium bitartrate crystals from forming in pure solution, it cannot be used to prevent argol formation in grape products. This is due to the fact that an unidentified inhibitor is sometimes present in grape juice which either limits or stops tartrase actvity. It will be necessary to identify this inhibitor and find a way to remove it before tartrase can be used successfully in grape products.

SUMMARY

1. An enzyme extracted from *Aspergillus versicolor* cultured on grape pomace was able to prevent potassium bitartrate crystals from forming in a pure solution of this salt.

2. The enzyme activity varied with the strain of *A. versicolor* that produced it and with the form in which tartrates were used. Tartrase was more effective against tartaric acid than against potassium bitartrate.

3. Tartrase activity was increased when iron, zinc, copper, molybdenum, magnesium, or manganese salts were present in the substrate. Of these iron was the most effective in increasing enzyme activity.

4. Enzyme activity was highest at an incubation time of two hours, at a temperature of 50° C, and at a 1 per cent potassium bitartrate concentration. Tartrase activity decreased as the pH of the substrate increased from 2.0 to 6.0. The optimum enzyme concentration was 0.20 per cent.

5. It was found that in the enzyme-catalyzed reaction, tartaric acid was decomposed to succinic acid.

6. Because of the presence of an inhibiter in some grape products tartrase activity is erratic and cannot be depended upon to prevent the formation of argol crystals in grape products.

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AN UNDESCRIBED FUNGUS CAUSING A ROOT ROT OF RED CLOVER AND OTHER LEGUMINOSAE [1]

J. W. Gerdemann

(WITH 7 FIGURES)

Many species of fungi are associated with red clover, *Trifolium pratense* L., root rots. One species which appears to be undescribed has been frequently isolated from diseased root specimens collected in Illinois and has been proven to be pathogenic (**3**). The fungus also has been isolated by the writer from dead red clover plants collected at Beltsville, Maryland, and Dr. R. G. Henderson reports having isolated it in Virginia from diseased roots of alfalfa, *Medicago sativa* L.

SYMPTOMS AND HOST RANGE

Pathogenicity tests were conducted in the greenhouse in steam sterilized, artificially infested soil. The mycelial and sclerotial growth from one agar slant was added to each 6-inch pot of soil at the time the seed was planted.

Emergence in infested pots of red clover and alfalfa was markedly reduced indicating pre-emergence damping-off. Post-emergence damping-off occurred in both species. Later the fungus penetrated the unwounded roots directly and caused a dark decay of the lateral root system and the taproot.

Preliminary experiments with this fungus indicate a wide host range within the Leguminosae. It has proven pathogenic on the following species: *Glycine max* (L.) Merr., *Lespedeza stipulacea*

[1] The writer wishes to express his appreciation to Dr. W. W. Diehl for assistance in checking literature and herbarium specimens; to Dr. Leland Shanor for helpful suggestions; and Dr. F. D. Lazenby of the Classics Department of the University of Illinois for the Latin diagnoses.

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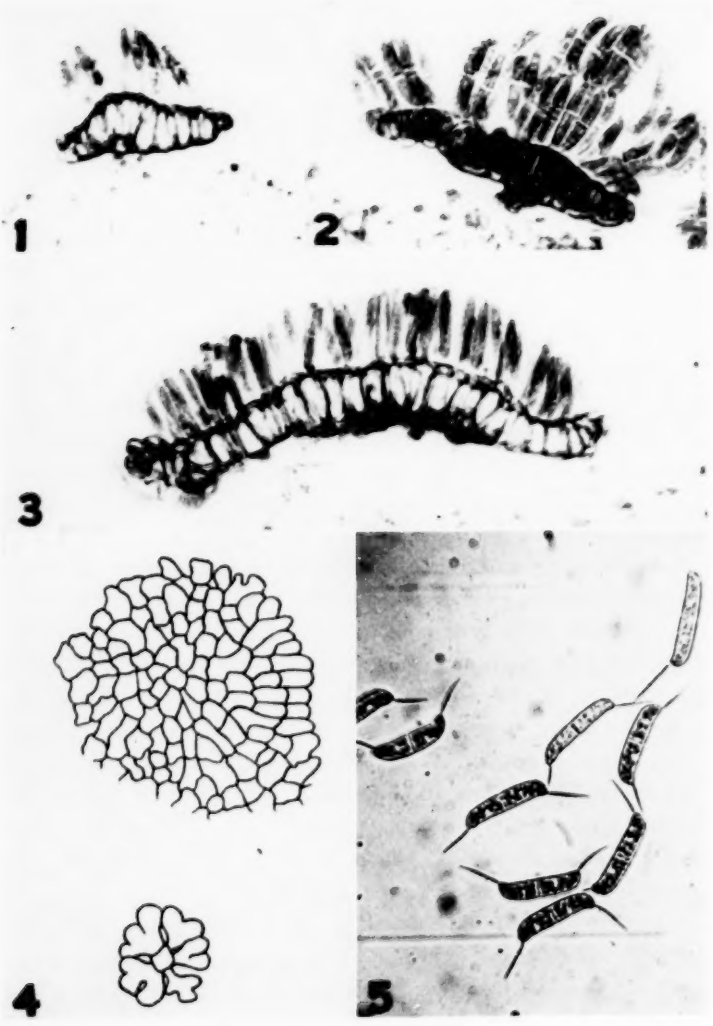
Maxim., *Lotus corniculatus* L., *Medicago sativa* L., *Melilotus alba* Desr., *Pisum sativum* L., *Trifolium hybridum* L., *T. incarnatum* L., *T. pratense* L. and *T. repens* L.

THE ORGANISM

Sclerotia are common in decayed red clover roots collected in the field and are produced in abundance on agar media. The imperfect fruiting stage has not been observed under field conditions but has been produced in the greenhouse on seedlings killed by the fungus. Fruiting has been obtained in culture on natural media sterilized with propylene oxide (**5**). Leaves and petioles of alfalfa, red clover, soybean, bluegrass or oats constitute a satisfactory medium for obtaining sporulation. The relation between the sclerotial stage and the imperfect fruiting stage was demonstrated by the use of single-spore cultures.

Sclerotia (FIG. 7) are black, spherical to fusiform in shape and may be as much as 1 mm. in length. The fruiting body (FIGS. 1, 2, 3, 4, 6) is superficial, yellow to dark brown in color, developing radially from a central cell to form a thin stroma, one cell layer thick, peltate, often fusing to form irregular plates as large as $200 \times 800\,\mu$ in diameter. Conidiophores are obsolete. Conidia are produced on the upper surface of the stromatic cells and are formed in linear rows in mucus. The mass constitutes a light yellow to brown head. The conidial walls are hyaline, but the cell contents are light yellow, often becoming brown as the spores age. Conidia (FIG. 5) are 1-septate when mature, allantoid in shape, with a filamentous seta at each end. The conidia measure 20–34.8 × 4.4–7 μ, the setae 8.7–18 μ in length.

The radial stroma often bears a superficial resemblance to the scutellum of members of the Micropeltaceae. The fruiting body might be defined either as a sporodochium or an acervulus. Employing the system of von Hoehnel (**6**) the superficial nature of the fruiting body would classify it as a sporodochium. Clements and Shear (**2**), however, regard the sporodochium as having developed from a compacting of hyphae and conidiophores, and state that the Tuberculariaceae, if properly limited, would comprise only those genera with long or branched conidiophores. They regard



FIGS. 1, 2, 3. Cross sections of acervuli of three sizes showing the one cell thick stroma upon which the spores are borne. × 455. 4. Camera lucida drawing of the spore-bearing upper surface of a young and an older acervulus illustrating the radial development from a central cell. Approx. × 550. 5. Conidia × 400.

the acervulus as a reduced fruiting body with short or obsolescent conidiophores and state that the distinction drawn by von Hochnel with respect to the insertion of the spore body may possess some validity, but is not a practicable criterion. The writer follows the view of Clements and Shear and regards this structure as most like an acervulus.

The setulate conidia of the legume fungus are suggestive of the genus *Discosia* (**4**). *Discosia*, however, differs in that the conidia

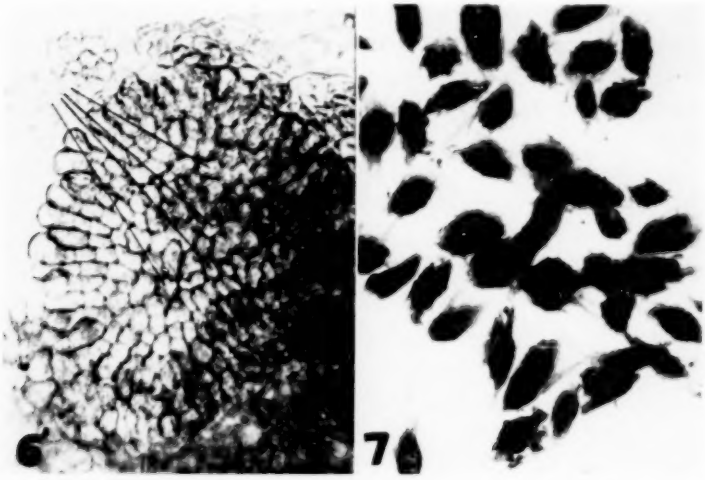


FIG. 6. Upper surface of an acervulus that resulted from the fusion of several acervuli at early stages in their development. Inked lines designate probable centers of origin. × 455. 7. Sclerotia from dead red clover roots collected in the field. × 15.

are 2- or more septate, the setae are borne to one side of the apex of the conidia, and the conidia are produced on conical conidiophores within black pycnidia. The 1-septate conidia of *Discosiella* (**11**) more closely resemble those of the legume fungus; however, *Discosiella* differs in that the conidia are borne on short hyaline conidiophores within completely enclosed black pycnidia that open by an irregular rupture. The conidia of *Dinemasporiella* (**10**) resemble those of the legume fungus but they are also borne within black pycnidia. *Pseudolachnea* (**9**) produces hyaline 1-septate

conidia with a seta at each end. However, they differ in that they are produced on branched conidiophores in black setulate pycnidia that are first enclosed but become cup shaped as they mature. *Dinemasporium* (**4**) produces hyaline 1-celled conidia that are setulate at each end. They are borne on linear conidiophores in cup-shaped black setulate pycnidia. The conidia of *Didymothozetia* (**8**) are 1-septate, 1-setulate at each end; but they are borne singly on ovoid conidiophores on globose hyaline sporodochia, and the setae are attached somewhat laterally. The legume fungus is distinguished from the above genera by the production of conidia directly from the cells of a superficial, one cell thick, radially developing stroma that is completely open at all stages. In addition the legume fungus has a prominent sclerotial stage.

The original description of *Pseudodiscosia* (**7**) refers to *Discosia*-like conidia produced directly from the cells of an acervulus. Later Buddin and Wakefield (**1**) reported conidiophores as present and their illustration of conidia bears little or no resemblance to conidia of *Discosia*. Grove (**4**) states: "It was said to resemble *Discosia* somewhat in its spores but that was a misconception." A genus having the characteristics of the legume fungus has apparently not been described and it becomes necessary to establish a new genus.

Leptodiscus gen. nov.

Sclerotia parva, nigra, sphaeralia vel fusiformia; acervuli superficiales, flavi vel fusci, scutiformes, qui e cellulo centrali radialiter orti stroma tenue efficiunt ad mensuram unius celluli crassum; conidiophora evanescentia; conidia lineari ordine in muco capitata; conidiorum parietes hyalini; conidia uno septo, una setula utrinque praedita.

Leptodiscus terrestris sp. nov.

Sclerotia nigra, sphaeralia vel fusiformia usque ad 1 mm. longa; acervuli superficiales, flavi vel fusci, scutiformes, qui e cellulo centrali radialiter orti stroma tenue efficiunt ad mensuram unius celluli crassum, et saepe inter sese nullo ordine concrescentes stroma efficiunt usque ad $200 \times 800\,\mu$ diametro; conidiophore evanescentia; conidia in summo stromate nata, lineari ordine in muco capitata; conidiorum parietes hyalini quod eis intercluditur

decolor vel flavum, interdum ad fulvum vergens; conidia cum adoleverint uno septo praedita, allantoidea, una setula utrinque praedita, 20–34.8 μ longa, 4.4–7 μ lata. Setae 8.7–18 μ longae.

The type is represented by a single-spore culture isolated from a diseased red clover root collected on the Agronomy South Farm of the Illinois Agricultural Experiment Station, Urbana, Illinois, in September, 1951. Material of the type has been placed in the Mycological Collection of the U.S.D.A. Bureau of Plant Industry, Soils and Agricultural Engineering, Beltsville, Maryland, and the Herbarium of the University of Illinois, Urbana, as Mycological Collection No. *31, 238*.

The fungus has been collected in different areas of Illinois, at Beltsville, Maryland, and Richmond, Virginia.

SUMMARY

An undescribed fungus causing damping-off and root rot of various Leguminosae is described and placed in a new genus, *Leptodiscus*. The genus is distinguished by the production of small black sclerotia, the superficial, thin radiate stroma upon which the spores are borne, and by the 1-septate conidia which have one filamentous seta at each end. The species has been designated as *Leptodiscus terrestris*.

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POLYOZELLUS MULTIPLEX AND THE FAMILY PHYLACTERIACEAE

ROKUYA IMAZEKI

The first record of the collection of *Polyozellus multiplex* in Japan was made by Z. Tashiro at Mt. Kirisima in Kyusyu, in September, 1920. A part of the collection was sent to C. G. Lloyd by A. Yasuda. Lloyd was intuitively impressed at its being a curious foliaceous pyrenomycete and described it under the name *Phyllocarbon Yasudai* Lloyd. It is needless to say, however, that he could not find a trace of perithecia. He also mistook the basidiospores for conidia. He then noted as follows: "There is no evidence of perithecia but no doubt they will be found at a later stage as most Pyrenomycetes have an earlier, conidial stage. The plant was sent as a Basidiomycete, but it is a safe proposition that no fungi of carbonous tissue are Basidiomycetes." The plant, however, is not carbonous at all, as stated by Lloyd.

There is preserved in the herbarium of the National Science Museum in Tokyo the duplicate type specimen of *Phyllocarbon Yasudai*. The present writer has previously noticed this misreference of Lloyd's in 1938,[1] in reporting the occurrence of this rare fungus in Japan from a specimen collected near Tokyo.

The fungus was described as *Cantharellus multiplex* by Underwood in 1899 and then transferred by Murrill to his new genus *Polyozellus*. From the time of the original collection, no one recorded the occurrence of this fungus from anywhere outside of the type locality until 1937, when Mounce and Jackson reported it from Quebec in Canada. They discussed it under the original name of Underwood. In 1938 Shope noted its occurrence in Colorado, but transferred it to the genus *Craterellus*. The present writer, as noted above, reported its discovery in Japan; he

[1] R. Imazeki. A rare fungus, Polyozellus multiplex (Underwood) Murrill [in Japanese]. Nat. Sci. and Museum, IX, **10**: 3-6. 1938.

proposed that the fungus should be separated from both *Cantharellus* and *Craterellus*, and Murrill's genus should be adopted for it. Recently (1947) Smith and Morse treated the fungus in their studies in "The Genus Cantharellus in the Western United States." They considered the fungus a unique species of *Cantharellus* and provided the new section *Polyozellus* for the species, not separating it from the genus.

The fungus is also very similar to *Craterellus* in general appearance, as Shope mentioned. Murrill had, however, separated the genus *Polyozellus* from *Craterellus* on the grounds that the fruit bodies of the new genus are compound whereas those of *Craterellus* are simple. Of this treatment, Shope considered that the compound fruit bodies and the rugose hymenium are in part typical of the genus *Craterellus*, and therefore considered the genus *Polyozellus* to be superfluous.

The present writer has a different opinion. Notwithstanding the external resemblance of the hymenophore, there is no genetic relation between *Cantharellus* or *Craterellus* and *Polyozellus*, but we may find a close resemblance between *Polyozellus* and the *Thelephora* group. The most important characteristic of *Polyozellus multiplex* is represented by the spore form. The subglobose and tuberculate spores are never found in any known species of the *Cantharellus* group. They are, however, representative of the *Thelephora* group. In addition to this, the present fungus has a dark violaceous or almost black color and a very strong odor, particularly in the dried condition. Such a deep color of the fruit body and the strong odor are very common in the *Thelephora* group, but never met with in the *Cantharellus* group. Thelephoric acid is said to be a characteristic pigment of the genus *Thelephora*, and is also contained in *Calodon ferrugineus*. The writer does not know whether this substance has a strong odor or not, but he is aware of many species among *Thelephora*, *Calodon*, and *Sarcodon* which are dark purplish brown in color and have a unique drug-like odor, especially in dried specimens. A similar odor is strongly given off by *Polyozellus multiplex*.

From these facts, the writer has thought of this present fungus as having affinity with the *Thelephora* group. Very recently,

Miss M. Sawada[2] proved the presence of thelephoric acid in *Polyozellus multiplex*. The writer's idea is now endorsed by this fact. In conclusion, the writer came to believe that *Polyozellus multiplex* has a natural relationship with the *Thelephora* group but not with the *Cantharellus* group, although the configuration of the hymenophore might suggest the opposite.

The writer believes that the configuration of the hymenophore should not be so highly estimated as of phylogenetic significance as is held by the conservative systematic mycologists. The most primitive form of the hymenophore is smooth and even in every taxonomic group. Then, if any morphological modifications take place, it is natural that they should tend to assume the warty, tuberculate, rugulose, venulose, or reticulate appearance, and finally attain the spinulose, porose, or lamellate form. It is logical to say that the configuration of the hymenophore does not represent a phylogenetic difference between the higher taxonomic groups, but merely a lower difference within each taxonomic group. There are many examples to verify the facts among many taxonomic groups. For example:

Tremellodon, Protohydnum, Protomerulius etc. in the Tremellaceae.

Lenzites, Irpex etc. in the Polyporaceae.

Hydnochaete, Hymenochaete, Cyclomyces etc. in other brown polypores.

Paxillus, Phylloporus etc. in the Boletales.

Filoboletus, Poromycena, Dictyopanus etc. in the Agaricales.

Thelephora, Calodon, Sarcodon, Boletopsis etc. in the Phylacteriaceae.

On these grounds, the genus *Polyozellus* should not only be thought valid, but also its natural taxonomic position should be transferred to the *Thelephora* group. For the group, Donk established the subfamily Phylacteroideae in the family Aphyllophoraceae in 1933, emending the conception of Phylacteriés of Patouillard. He included in his subfamily three tribes—Thelephorinae,

[2] Sawada, M. Studies on pigments in fungi (I). On the distribution of thelephoric acid in fungi [in Japanese with English resume]. Journ. Jap. Forestry Soc. **34**: 110–113. 1952.

Hydnelleae, and Boletopseae. The writer agrees with his idea, but he proposes to give a further emendation to this group, raising it to the rank of family and adding a new tribe Polyozelleae.

Family **Phylacteriaceae** nom. nov.

Phylacteriacées Konr. & Maubl., Icon. Sel. Fung. **6**: 494. 1937.

Phylactéries Patouillard, Ess. Tax. 117. 1900—Bourdot & Galzin, Hym. Fr. 446. 1927.

Phylacteroideae Donk, Rev. Nied. Homob.-Aphyll. II, in Meded. Bot. Mus. Herb. Univ. Utrecht **9**: 24. 1933.

Hydnaceae Fries *et al.*, pr. p.

Thelephoraceae Fries *et al.*, pr. p.

Fructificatio resupinata, effuso-reflexa, pileato-sessilis vel stipitata, in forma sessili effuso-reflexa vel dimidiata, in forma stipitata umbelliformis vel ramoso-clavariformis vel polypileata (ut in *Grifola frondosa*); stipes simplex sed ramosissimus in *Thelephora* et *Polyozello*, contextus hypochnoideus, carnosus, coriaceus, generatim fusco- (brunneo- vel nigro-) coloratus, per exceptionem albus, exsiccatus saepe fortiter odoratus; hymenophorum laeve, tuberculatum vel spinulosum, raro porosum rugulosumque; sporae hyalinae, generatim coloratae, subglobosae et angulatae vel verrucosae; hyphae ad septa nodosae.

The Phylacteriaceae thus emended is a very natural group, but it is not easy to express descriptively the clear conception of this family, because the only characteristic common to all the species of the family is the globose and verrucose or tuberculate character of the spores. The stichobasidial type is regarded by Donk as an important character of the family. In *Thelephora*, the basidia are stichobasidial and arranged protohymenially. But this feature is not constant in other tribes. Spores are usually colored but sometimes hyaline. The presence of thelephoric acid is one of the noteworthy characters, which has been investigated in many species of *Thelephora* and also in *Calodon ferruginosus*. As noted above, Miss M. Sawada recently tested its presence in *Sarcodon aspratus*, *Calodon gravcolens*, and *Polyozellus multiplex*. The writer is sure that this pigment will be found to occur in many other species of this family in the future. The strong drug-like odor is very

frequently met with in *Thelephora*, *Sarcodon*, *Calodon*, and *Polyozellus*, which are deep-colored species.

The family is divided into the following four tribes.

Tribe 1. THELEPHORINAE Donk, Meded. Bot. Mus. Herb. Univ. Utrecht **9**: 25. 1933.
Phylacteriacées Konrad et Maublanc, Incon. Sci. Fung. **6**: 494. 1937.

Fructificatio resupinata in generibus primitivis (*Caldesiella*, *Tomentella* et *Kneiffiella*), pileato-stipitata vel ramoso-clavariformis in *Thelephora*; contextus floccosus, membranaceus vel coriaceus; semper obscuriter coloratus; hymenophorum laeve vel tuberculosum, raro spinulosum (in *Caldesiella*).

Genera included: *Kneiffiella* Karsten; *Tomentella* (Pers.) Pat.; *Caldesiella* Sacc.; *Thelephora* Fries (*Phylacteria* Patouillard).

Since the time of the publication of the "Essai taxonomique" by Patouillard, the French mycologists have generally followed him in the arrangement of higher fungi. There is much disagreement in the interpretation of certain Friesian genera among the modern taxonomists of France and other countries. The genus *Thelephora* is one of them. The name *Thelephora* sensu Patouillard is applied to *Stereum pallidum* (*Thelephora pallida* Fr.) and its allies by some, while it is used for the group including *Thelephora terrestris* by others. Patouillard and his followers considered that the genus *Thelephora* was based on *Thelephora pallida* Fr., and he gave, therefore, the name *Phylacteria* to *Thelephora terrestris*, which has brown, verrucose spores and papillate hymenophore. On the other hand, Burt and Donk designated *Th. terrestris* as the type species of the genus *Thelephora* Fr. sensu Fr., 1821, and maintained that *Thelephora* should be used for the group with brown verrucose spores and papillate hymenophore, as had been done for a long time.

To avoid confusion in nomenclature, the writer wishes to use the name *Thelephora* in the sense of Burt, Donk, and most other mycologists, if there occurs no insuperable obstacle to doing so. As Donk noticed, Fries gave for *Thelephora* the following diagnosis and notes in his Systema Orbis Vegetabilis (1825): "Hymenium subpapillosum, inaequabile. Sporidia quaternata, fuscopurpurea" and "Cum hujus generis typo, *Thelephorae terrestri*,

nomen genericum tribuerit Ehrhartus, sensu primi determinatoris servare coactus fui." These words represent very clearly the concept of the genus *Thelephora* sensu Fries, in the meaning customarily used. Fries, moreover, apparently indicated *Thelephora terrestris* [Ehrh.] Fries as the type species of the genus.

It is not only difficult but nearly impossible to secure the real idea held by Fries directly from such an extremely complicated, heterogeneous genus as *Thelephora* as presented in the Systema Mycologicum. However, Fries's intention is clarified in the Systema Orbis Vegetabilis, as shown above. From the etymological standpoint, the name *Thelephora* means papillate hymenophore which is one of the representative characteristics of the "*terrestris*" group.

From these facts it does not seem illegal nor illogical to consider that the kernel of the genus *Thelephora* Fries of 1821 was *Th. terrestris* and its allied species, but not others.

Tribe *2.* HYDNELLEAE Donk, Meded. Bot. Mus. Herb. Univ. Utrecht **46**. 1933.
Sarcodontées Konrad & Maublanc, in Fam. Hydnaceae, Icon. Sel. Fung. **6**: 492. 1937.

Fructificatio stipitata, terrestris; stipes simplex; pileus carnosus vel coriaceus; hymenophorum distincte spinulosum.

Genera included: *Calodon* Quélet (*Hydnellum* Karst., *Phellodon* Karst., *Hydnum* Auct. plur., pr. p.); *Sarcodon* Quélet (*Hydnum* Auct. plur., pr. p., *Phaeodon* Schroet.).

Tribe *3.* **Polyozelleae** trib. nov.

Fructificatio terrestris merismatoidea ut in *Grifola frondosa;* contextus carnoso-mollis, in exsiccato fragilescenti rigidescens, nigricante coloratus; hymenophorum rugulosum ut in *Cantharello.*

This tribe contains only a monotypic genus *Polyozellus* Murrill, N. Am. Flora **9**: 171. 1910.

Polyozellus multiplex (Underwood) Murrill, N. Am. Flora **9**: 171. 1910.
Cantharellus multiplex Underwood, Bull. Torr. Bot. Club **26**: 254. 1899; Smith and Morse, Mycologia **39**: 502. 1947.
Craterellus multiplex Shope, Mycologia **30**: 373. 1938.
Phyllocarbon Yasudai Lloyd, Myc. Writ. **6**: 1066, f. 2003. 1921.

Nom. jap.: Karasu-take, Karasu-maitake.

Distr.: U. S. (Maine, Oregon, and Colorado); Canada (Quebec, British Columbia); Japan; and Korea (according to Mr. Iwade).

Materials examined: Specimens of Herb. Nat. Sci. Mus. (Mt. Kirisima, leg. Z. Tashiro, Sept. 5, 1920, **type** of *Phyllocarbon Yasudai* Lloyd; Asakawa, Pref. Tokyo, leg. R. Imazeki, Oct. 25, 1936); Specimens of Herb. For. Exp. St. (Asakawa, Pref. Tokyo, leg. S. Toki, Oct. 17, 1947 and May 19, 1948).

Tribe 4. BOLETOPSEAE Donk, Meded. Bot. Mus. en Herb. Rijks Univ. Utrecht **9**: 64. 1933.

Fructificatio stipitata, carnosa; hymenophorum porosum. Genus contained: *Boletopsis* Fayod (*Boletopsis leucomelas* (Pers. ex Fr.) Fayod).

The writer wishes to express his heartiest thanks to Dr. D. P. Rogers, who carefully read this paper and gave very valuable suggestions and advice, and also gave him facilities to refer to Fries's Systema Orbis Vegetabilis which was not available to him.

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ON THE STATUS OF THE GENERIC NAMES PYRENOPHORA AND PLEOSPORA

L. E. Wehmeyer

The genus *Pyrenophora* has been variously interpreted. It has been recognized as distinct from *Pleospora* by some investigators and united with this genus by others. However, when the two genera have been united, the genus name *Pleospora* has been used by nearly all authors. Inasmuch as *Pyrenophora* Fries (**1849**) has priority over *Pleospora* Rab. (**1854**), this is incorrect usage and would involve wholesale name changing. It is of import, therefore, to determine the status of these two names.

The genus *Pyrenophora* was described by Fries (**1849**), as follows:

"Nucleus serotinus, grumoso-gelatinous, in stromate ceraceo indurato (Sclerotiodea), perithecii vices gerent, immersus, ostiolo tarde prominule.

"Asci firmi, sporis opacis multiseptatis Berk. Suppl. Vol. VI. tll, f. 8."

Singulare et affinitatis incertae genus, cui plura exotica habemus affinia. Cfr. Dothiorum.

Three species were given as follows:

1. *Pyrenophora paradoxa* Fr. 1 (nucl. pallideus) S. M. p. 549.
2. *P. inclusa* (Kunze sub. Sclerot.) 1.
3. *P. phacocomes* Reb. 1, 2, B. n. 185 Grev. Scot. t. 69.

Through the kindness of J. A. Nannfeldt it has been ascertained that there are only two collections of these species, one of *P. inclusa* and one of *P. phaccomes* in the Fries Herbarium. Whether or not these are holotype material is doubtful, but they are, apparently, the only lectotype material available, or in existence.

P. paradoxa was previously described (Fries **1823**) as *Dothidea paradoxa*, as follows: "subcaespitosa, nuda, elongate, difformis, rugosa, atra, intus alba." It is given as "rarissima" and on de-

nuded *Salix* wood. There appears to be no specimen in existence, and the species has been dropped from the literature.

P. inclusa was based on *Sclerotium inclusa* Schm. & Kze. (Deut. Schwamme *137*) and described by Fries (**1823**) as on poplar leaves. A collection in the Fries Herbarium consists of a single poplar leaf with a number of sterile sclerotia similar in appearance to *Sclerotium bifrons*. These sclerotia are circular, disc-shaped, black, with wrinkled upper exposed surface and are 800–1000 × 400–500 μ. There is an outer blackened crust and an inner hyaline parenchymatic cortex with some of the cells containing a yellow resin-like material, but beyond this there is no differentiation and there are no setae nor any tomentum.

Pyrenophora phacocomes (Reb.) Fr. is the species which is usually cited in the literature as the type species of *Pyrenophora*, because the two previous species were poorly known. *Sphaeria phacocomes* was described by Rebenitsch (**1804**) as follows: "simplex, sparsa, epiphylla, globosa hemisphaeriis, utrinque folii pagina pilis erecto-divergentibus obsitis. R. Tab. 1, fig. 4a, 4b. In *Holci mollis* foliis languescentibus. Autumno.

"Sphaeria atra duriuscula globosa, intus albida, utrinque folii pagina pilis oblita. E. longinquo *Dematium* effe videtur at accuratius adspecta et transversim perscissa ab eo facile diagnoscitur."

His figures are merely rough sketches of the type of setose sclerotia usually associated with this species. There is a collection of this species in the Fries Herbarium consisting of five or six stems and leaves of some grass which contain a number of sclerotial ascocarps typical of such species as *Pyrenophora trichostoma* (Fr.) Fck. and *P. phacocomes* (Reb.) Fr. They are 350–500 × 250–400 μ in diameter, somewhat depressed-globose, buried in the leaf tissue, finally erumpent-superficial, and bear a crown of dark brown, tapered, sparingly septate, stiff, setose hyphae, up to 70 μ in length and 7–12.5 μ in diameter. There is an outer blackened wall or rind and an inner hyaline parenchymatic cortex, but all of the 12–15 fruit bodies examined were completely sterile.

From the preceding it can be seen that all of the Friesian type material consisted of sterile sclerotia (and probably also all pre-

Friesian material cited by Fries), and according to Art. 57, as revised at the Stockholm Congress, the name *Pyrenophora* would refer to this sterile state and not be valid for an ascus or perfect genus.

Fries's description also refers only to sterile sclerotia, except that portion which is placed in quotation marks, and apparently refers to the Berkeley (**1841**) reference, which figures spores and asci. As a matter of fact, Berkeley's figures do not represent the asci and spores of Fries's sclerotial fungus, as will presently be shown, and if this is accepted as a part of the description of the type of *Pyrenophora* then this type, *P. phacocomes,* consists of a mixture of "two or more entirely discordant elements" as mentioned in Art. 64 of the International Rules, and the only choice of a "satisfactory type" would be the sclerotial stage of Fries's specimens of *P. phacocomes.*

In the reference mentioned by Fries, Berkeley (**1841**), in a discussion of *Diplodia viticola* Desm., referring to the method of cavity formation in the stromatic pycnidium, says: "This is the case with certain species of *Sphaeria,* which will hereafter probably constitute a new genus. Amongst them is the curious *S. phacocomes,* which I have found in fructification and of which I give a figure." The figure (Pl. 11, fig. 8) presents a few asci and spores, which have three transverse and one or more vertical septa, but appear much smaller than those typical of the perfect stage of Fries's sclerotia. Berkeley (**1860**) listed the genus *Pyrenophora* and gave the spores as "multiseptate" and listed one species, *P. phacocomes* Fr.

The earliest collection of *Sphaeria phacocomes* Berkeley and Broome that the writer has been able to locate is found on two sheets from the Broome Herbarium, labelled *Sphaeria phacocomoides,* in the British Museum of Natural History. The data on the sheets give the fungus as being on *Alisma plantago,* Rudloe, 6/1, — 43 and on one sheet there is a notation "Annals N. H. vol. 6 (*Pyrenophora* Fr.) (foliicola)" and figures of spores similar to those mentioned above. Although this collection was apparently made after the note was published in the Notices of British Fungi, it seems to be authenticated by the authors. This collection is of

Pleospora vulgaris Niessl. It has perithecia which are 150–200 μ in diameter and with no setae. The asci are 65–85 × 8.5–13.5 μ and the spores are 3–5-septate, oblong-ellipsoid, constricted at the septa, with vertical septa in the central cells only, and measure 15–18 × 5.5–6.5 μ. Fries's reference to the spores as "opacis" would also suggest this species rather than the ascospores of *P. phacocomes*, which are pale yellow for some time before maturity.

The reference to Greville (**1823–29**), by Fries and Berkeley, is the description of *Cryptosphaeria capillata*. He figures setose fruiting bodies similar to those of Fries's *P. phacocomes*, but again states: "I have not been able to discover the sporules" and refers to Nees von Essenbeck's (**1817**) *Sphaeria capillata*, who figures "ascus granules" with three septa.

Cesati and de Notaris (**1861**) listed *Sphaeria phacocomes* Berk. & Br. under their genus *Pleospora*. Niessl (**1876**) described *Pleospora phacocomes* Ces. & de Not. and based it upon *Sphaeria phacocomes* Berk. & Br. (Brit. Fung. Nr. *207*). Niessl gave the species as upon *Vitis*, probably because it was discussed under *Diplodia viticola* by Berkeley, and also on Umbelliferae. He said the spores were dark-colored (as given by Fries), 5-septate, and of the form of *Pleospora media*, which agrees with the material in the Broome Herbarium. Saccardo (**1883**) transferred this species to *Pyrenophora*, recognized it as distinct from *P. phacocomes* (Reb.) Fr., named it *P. phacocomoides* Sacc. and properly recognized *P. phacocomes* as based upon *Sphaeria phacocomes* Reb., but incorrectly referred the *Pyrenophora* binomial to himself and the *Pleospora* one to Fries instead of vice versa.

Berlese (**1900**) figured the spores of these two species, showing the same differences. *Pyrenophora phacocomoides* (from Niessl's material) shows (**1900**, Pl. 53, fig. *2*) the spores of *Pleospora vulgaris* but setose perithecia. *Pyrenophora phacocomes* (Reb.) Fr. (**1900**, Pl. 54) shows very large, 6–7-septate ascospores and the large setose perithecia as found in sterile collections of this species.

From the preceding, it is obvious that Berkeley and Broome's *Sphaeria phacocomes* is not the same as that of Rebenitsch, but is the same as *Pleospora vulgaris* Niessl, and that it is not the ascus

stage of the large setose sclerotia of Rebenitsch's figures and Fries's collection. It also appears that the genus name *Pyrenophora* must either be considered as a form genus name (near *Sclerotium*) because of the sterile nature of the type material, or else be discarded, if Fries's reference to Berkeley's description of spores is accepted, for the description then refers to two discordant elements.

THE GENUS PLEOSPORA

There are also certain obstacles in the way of acceptance and typification of the genus name *Pleospora*, which should be cleared up. The genus name *Pleospora* was first used by Rabenhorst, upon a series of exsiccati (Herb. Myc. II, *547a-c*) issued as varieties of a species *Pleospora herbarum* Rab. On the first exsiccatus (*547a*) are the following notes:

Pleospora Rabenh. Sphaeriacerum nov. genus. Sphaeriae sp. (herbarum) Autt.
Pleospora herbarum Rab. var. *Allii* Rab. Mspt. (*S. Allii* Rab. Herb. Myc. I, 838—*S. herbarum* var. *Allii* Desm.) *Allium*, Doenitz, leg. Fischer.

The genus *Pleospora* has generally been referred to Rabenhorst on the basis of this exsiccatus. As a matter of fact, this does not constitute either a species or generic description, and although the name *Sphaeria herbarum* (Autt.) is mentioned, it is obvious that Rabenhorst was not sure of this synonymy.

The type collection of *Sphaeria herbarum* Fr., quoted by Fries (**1823**, *2*: 511) as Scler. Suec. No. *38*, has proven to contain only a small *Phoma*, both in the copy in the Uppsala Herbarium of Fries (kindly examined by J. A. Nannfeldt) and in that in the Farlow Herbarium (examined by the writer). The Persoonian type of *Sphaeria herbarum* var. *tecta*, quoted by Fries (**1823**, *2*: 511), was kindly loaned by H. J. Lam of the Leiden Rijksherbarium, and proved to be a species of *Ophiobolus*. It is obvious, therefore, that the use of the epithet "*herbarum*" for Rabenhorst's exsiccatus was a misdetermination, and that *Pleospora herbarum* Rab. refers either to a *Phoma* (if one uses the Friesian type) or to an *Ophiobolus* (if one chooses to go back to the Persoonian type). In a later exsiccatus, of the same year (Herb.

Myc. II, *731*) of *Sphaeria Typharum*, Rabenhorst refers to *Sphaeria scirpicola* in the following words: "sporis multicellularibus (transv. et longitudinaliter septatis) gaudet, qua de causa PLEOSPORA VERA et minime Sphaeria." This again is scarcely to be called a generic description.

The first properly published description of the name *Pleospora* was by Cesati and de Notaris (**1861**). Their description as "Sporidia ampla; plurilocularia, loculis transvers seriatis" would also exclude *Pleospora herbarum* (Fr.) Rab. as typified by Scler, Suec. No. *38*. These authors list three generic synonyms, fourteen species of *Pleospora* and five species of *Sphaeria* under this generic name. The sixth species of Cesati and de Notaris is *Pleospora Allii* which is based upon Rabenhorst's *P. herbarum* var *Allii*, which in turn is based upon *Sphaeria Allii* Rab. (as issued in Herb. Myc. I, *838*).

Inasmuch as the name *Pleospora herbarum* is attached to a Friesian type of the genus *Phoma* (or a Persoonian type of the genus *Ophiobolus*), as Rabenhorst's original use of the generic name *Pleospora* did not include a generic or specific description, and inasmuch as Cesati and de Notaris' sixth species, under the first proper description of the generic name, refers to Rabenhorst's original exsiccati (Herb. Myc. I, *838* and II, *574a*), which he obviously meant to be the basis of the genus *Pleospora*, it is proposed that the genotype of this genus (*Pleospora* Rabenh. ex Ces. & de Not.) be conserved as *Pleospora Allii* (Rab.) Ces. & de Not., with Herb. Myc. I, No. *838* as the lectotype collection of the species.

Such a disposition, as outlined, will involve only one name change, the substitution of *Pleospora Allii* (Rab.) Ces. & de Not. for *P. herbarum* (Rab.) Fr., and will establish the genus *Pleospora* on a broad enough basis to include species formerly placed in the (form ?) genus *Pyrenophora*, if so desired.

The fact remains that there are a group of species related to *Pleospora trichostoma* which have certain characters in common which, together, distinguish them from most other species of *Pleospora*. These characters are the sclerotial character of the perithecium with a thick stromatic wall, the presence of a crown

of seta-like hyphae about the ostiole, the large pale yellow-brown ascospores and the presence of a *Helminthosporium* conidial stage. The difficulty lies in the fact that no one of these characters is a constant difference. *Pleospora herbarum* and other species may have large sclerotial perithecia; the crown of setae is common in other species of *Pleospora;* some of the *P. trichostoma* group, such as *P. macrospora* on *Hierochloe,* may lack such setae; and there are, again, strains of *P. trichostoma* which do not produce *Helminthosporium*-like conidia.

The name *Scleroplea* might be available for this group, if considered a separate genus. Saccardo (**1883,** p. *277*) used this name for a subgenus of *Pleospora,* with sclerotioid perithecia. He obviously considered the setose ostiole as the distinguishing character of *Pyrenophora.* Oudemanns (**1901**) raised it to generic rank and von Höhnel (**1907,** p. *635*) considered it as differing from *Pyrenophora* in the lack of the ostiolar setae. The first species mentioned by Sacardo under his subgenus *Scleroplea* is *Pleospora nuda* (Cke.) Sacc. The writer has examined three portions of Cooke's type collection of *P. nuda.* They all show black sclerotial areas 300–1000 μ long, on leaves, which are entirely sterile. In the specimen at the Kew Herbarium, masses of black toruloid hyphae were seen on these black spots; the packet in the Riksmuseet showed only an accompanying *Ascochyta.* A copy in the Farlow Herbarium yielded a few perithecia with ellipsoid, muriform spores, *22–27* × 10–11 μ and 5–7-septate. This was *P. media* or *P. herbarum.* Cooke (**1879**) in his original remarks says, "there are no true perithecia, the cells surrounding the perithecial cavity are brown, globose, and readily separable." His description apparently includes a mixture of fungi. Oudemans placed his *S. cliviae* as his first species. The writer has not seen this fungus but the description and figures of Oudemans (**1901**) again suggest *P. herbarum.* It seems that if this "*Pyrenophora*" group is to be recognized, a new generic name must be created, but inasmuch as much more needs to be known concerning the details of perithecial development before the true relationships and proper disposition of species can be determined in this group, such a creation at this time does not seem practicable.

The three following species are all closely related and of this "*Pyrenophora*" type, and they are all found upon grass hosts.

PLEOSPORA TRICHOSTOMA (Fr.) Ces. & de Not. Schema sfer. ital. 217. 1861.

This species has been discussed previously (Wehmeyer **1949**). It occurs on a large variety of grass genera and has 3-septate ascospores which show a wide range in size (35–60 × 13–23 μ) and has been described under a number of species names. Both Fuckel (**1869**) and the Tulasnes (**1863**) report the spores as 3–4-septate but the writer has not seen such spores with more than three septa and they are probably unusual, except, perhaps, upon germination.

PLEOSPORA PHAEOCOMES (Reb.) Wint. in Rab. Krypt. Fl. Deutsch. etc. **1**(2): 521. 1887.

Sphaeria phaeocomes Reb. non Berkeley, Prodr. Fl. Neomarch. 338. 1804.

Pyrenophora phaeocomes (Reb.) Fr. Summa Veg. Scand. 398. 1849.

This species differs in the larger spores (40–100 × 20–41 μ) which have 4–6 (–7) septa. The perithecial sclerotia of this species remain sterile for a long period of time and are so found in many collections and exsiccati. Fuckel (**1869**) seems to have been the first to describe the spores of this species, which he states are 3-septate at first, then multiseptate and muriform. His figure (Pl. 6, fig. 31) shows a 3-septate spore with appendages and without vertical septa, presumably a young spore (?). Berlese (**1901**, Pl. 54) gives good figures of the spores of this species. Rebenitsch, Fuckel, Winter and Berlese all report this species on *Holcus*. A collection of this species, with 6-septate spores, on *Anthoxanthum* was sent to the writer by Dr. L. Holm from Sweden, and Müller reports it upon *Calamagrostis* from Switzerland.

PLEOSPORA MACROSPORA Schroet. Hedw. **2**: 153. 1882 (Jahresber. d. Schles. Ges. d. Vaterl. Cultur. 1880).

Clathrospora macrospora (Schroet.) Nannf. Mitt. Bot. Inst. Techn. Hochschuhle Wien **8**: 30. 1931.

This species appears to be common in northern Europe on *Hierochloe*, but has also been found by Mr. C. B. Kenaga, near

Ann Arbor, Michigan, on *Poa sp.* It is very similar to *Pleospora trichostoma* in its 3-septate spores, which, however, are toward the lower end of the size range (30–45(–50) × 10–18 μ). The perithecia are smaller than in *P. trichostoma* and are almost smooth or with a few short hair-like setae.

Pleospora scirpicola (DC.) Karst. and *P. typhaccola* (Cke.) Sacc. are also similar to the above three species, and the spores of both show an irregular flattening in one plane, which is also true to a lesser degree in the spores of *P. macrospora.*

In the writer's opinion, therefore, the following conclusions should be reached:

1. That *Pleospora Allii* (Rab.) Ces. & de Not., with Rabenhorst's Herb. Myc. I, *838*, of *Sphaeria Allii*, as the type specimen, be conserved as the lectotype of the genus *Pleospora.*

2. That the genus name *Pyrenophora* be removed from the Ascomycetes to the Fungi Imperfecti as a form genus name.

3. That until the proper taxonomic limits of the group of species about *Pleospora phaeocomes* can be determined, they be retained as a sub-group of the genus *Pleospora.*

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PUCCINIOSTELE, A GENUS OF THE RUST FUNGI [1]

GEORGE B. CUMMINS AND M. J. THIRUMALACHAR

(WITH 1 FIGURE)

This paper presents information concerning the morphology of the genus *Pucciniostele* Tranz. & Kom. (*Klastospora* Diet.). The four species thus far described are *P. clarkiana* (Barcl.) Diet., *P. mandschurica* Diet., and *P. sydowii* Liou & Wang on species of *Astilbe*, and *P. hashiokai* (Hirat. f.) Cumm. on *Impelopsis*. An additional species, *P. philippinensis*, on *Astilbe* is described below. All are Asiatic in distribution.

CHARACTERISTICS OF THE GENUS

SPERMOGONIA AND AECIA. The spermogonia are subcuticular, applanate or lenticular in shape, and with a flat or nearly flat sporogenous layer. In size they vary from 40 to 60 μ in height and 100 to 200 μ in diameter with no marked variation between species. Some hypertrophy results from infections on *Astilbe*. The aecia are caeomoid, but in *P. philippinensis* there is present what may be a rudimentary peridium. Spore-like cells adhere to the epidermis in a peridium-like manner, are usually brownish, and have irregular markings of a labyrinthiform nature. The aeciospores are verrucose with small rounded warts and are provided initially with intercalary cells which are displaced as the spores mature. For the species on *Astilbe* the aecia at first surround and later displace the spermogonia since they occupy areas

[1] Cooperative investigations between the Purdue University Agricultural Experiment Station and the Division of Mycology and Disease Survey, Bureau of Plant Industry, Soils, and Agricultural Engineering, United States Department of Agriculture, Journal Paper No. 660, of the Purdue University Agricultural Experiment Station. Contribution from the Department of Botany and Plant Pathology.

several millimeters in length. Aecia are not known for *P. sydowii* and *P. hashiokai*.

UREDIA. Previous to this study uredia were not recognized, *P. clarkiana* and *P. mandschurica* being considered, and probably rightly so, as *opsis*-forms. However, *P. philippinensis* develops uredia as does also *P. hashiokai*, but the two are unlike. In *P. philippinensis* the uredia are provided with a uniseriate peridium composed of more or less cubical cells which are firmly united. The peridium adheres closely to the epidermis of the host but can be separated off in sizeable sheets. Like the aeciospores, the urediospores are catenulate and verrucose. In *P. hashiokai*, however, the uredia have peripheral paraphyses as in *Phakopsora* and the echinulate spores are borne singly. The inclusion in one genus of species having such diverse uredia is open to question.

TELIA. *Pucciniostele* is unique in that two kinds of telia, primary and secondary, have been reported and do occur in the species on *Astilbe*. This has been confirmed and illustrated by Koursanov, Zeshinskaja, and Klushnikova (**4**) who studied *P. mandschurica* cytologically. The primary telia develop in the base of the aecia and completely displace the aeciospores. The primary teliospores are usually in the form of tetrads (FIG. 1, A) and arise from the same basal cells as do the aeciospores. Consequently, a single linear series may have chains of aeciospores apically and chains of teliospore tetrads basally. In later stages the sorus may contain only teliospores chains, which are sufficiently obvious to be observed with a hand lens. Lateral adherence between chains is not great but adherence between tetrads is strong. While the tetrad is commonest, teliospores may be unicellular or bicellular. Koursanov *et al.* (**4**) report that the teliospores are initially binucleate but become uninucleate by nuclear fusion. Germination of the primary teliospores has not been observed and their role in the life cycle is unknown.

Secondary telia develop later in the season and not in close association with the aecia. In *P. philippinensis*, as also in *P. hashiokai*, they are associated with the uredia. As was originally emphasized by Barclay (**1**), the telia have a superficial resemblance to those of *Colcosporium* in that they are golden and waxy in

appearance when immature but becomes nearly brown when mature. The telia consist of subepidermal crusts (FIG. 1, B) of unicellular teliospores produced catenulately. Lateral adherence of the chains is not great, and as the sorus reaches maturity they separate in varying degrees and fall from the sorus, leaving only the bounding ruptured epidermis of the host. Germination of the secondary teliospores has not been observed. While obscure, there is some evidence that there may be two or three germ pores in the species on *Astilbe* and this is certainly true of *P. hashiokai*.

THE SPECIES OF PUCCINIOSTELE

PUCCINIOSTELE CLARKIANA (Barcl.) Diet. (Engler's Bot. Jahrb. **27**: 564. 1899) occurs on *Astilbe congesta*, *A. japonica*, *A. kiusiana*, *A. longicarpa*, *A. macrophylla*, *A. rivularis*, and *A. thunbergii* in China, India, and Japan.

The species develops spermogonia, caecomoid aecia with the aeciospores measuring 16–24(–26) × (21–)24–32(–35) μ and having an apical wall 4–10 μ in thickness, primary telia in the aecia, and secondary telia. Uredia are not known.

PUCCINIOSTELE MANDSCHURICA Diet. (Ann. Mycol. **2**: 21. 1904) occurs on *Astilbe chinensis* and *A. microphylla* in China, Japan, Korea, and Manchuria.

The species forms spermogonia, caecomoid aecia with the aeciospores measuring (14–)17–24 × (18–)22–28(–30) μ and having the apical wall uniform or thickened to 4 μ, primary telia in the aecia, and secondary telia. Uredia are unknown.

PUCCINIOSTELE SYDOWII Liou & Wang (Chinese Jour. Bot. **1**: 81. 1936) occurs on *Astilbe* sp. in India. Only telia are reported. It is doubtful if this species differs from *P. clarkiana*.

Pucciniostele philippinensis sp. nov.

Spermogoniis amphigenis, subcuticularibus, lenticularibus, 45–60 μ altis, 120–200(–275) μ latis, sine paraphysibus. Aeciis caecomoideis, hypophyllis vel caulicolis, magnis, frequenter confluentibus, rotundatis vel elongatis et usque ad 2 cm longis, aurantiacis; aeciosporis variabilibus sed plerumque oblongis vel oblongo-ellipsoideis, (17–)20–26(–30) × (29–)32–40(–43) μ, membranis 2–3 μ cr. ad apicem 8–15(–20) μ cr., moderate verrucosis, hyalinis. Urediis sparsis, hypophyllis, aureis, peridio ex cellulis cuboideis, 12–15 μ

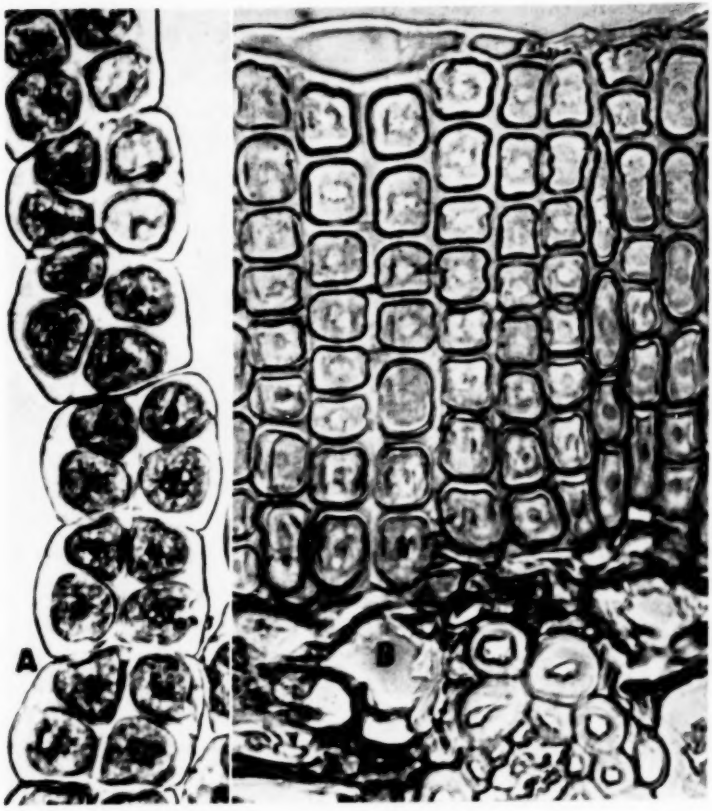

FIG. 1. Types of teliospores produced by the species of *Pucciniostele* on *Astilbe*. A. Primary teliospores of *P. clarkiana*. This spore form develops in aecial sori. The characteristic tetrads of spores do not adhere laterally but are attached vertically, resulting in chains of tetrads. B. Secondary telia of *P. mandschurica* in section. These telia occur later in the season than the primary telia and are not associated with the aecial infections. The unicellular spores are strongly adherent vertically but the chains tend to separate laterally at maturity. Both kinds of telia are known in *P. clarkiana*, *P. mandschurica*, and *P. philippinensis*. Germination has not been observed in either type. × 800.

diam., flavidis, membranis 1.5–2 μ cr. instructis; urediosporis catenulatis, oblongis vel oblongo-ellipsoideis, 17–24 × 26–31 μ, membranis hyalinis, 2 μ cr. vel ad apicem 4 μ cr., moderate verrucosis. Teliis primariis ex aeciis ortis, teliosporis primariis irregularibus plerumque ex 4-cellulis compositis,

19–22 × 26–32 μ, cellulis singulis plus minusve cuboideis, pallide aureis; teliis secondariis sparsis, hypophyllis, subepidermalibus, aureis, rotundatis, 0.1–0.6 mm diam., teliosporis 4–8 in catenas cylindraceas connexis, sporis individuis cuboideis, 13–14 × 10–15 μ, membranis pallide flavidis, 1–1.5 μ cr.

On leaves, petioles, stems, and inflorescence of *Astilbe philippinensis* Henry in the Philippine Islands. The type of the species is Clemens No. *51787* collected on Mt. Santo Tomas, Benguet Prov., Luzon, Mar. *26*, 1935.

P. philippinensis differs from *P. clarkiana* and *P. mandschurica* in several respects. Uredia apparently occur only in *P. philippinensis*. It has the largest aeciospores and the greatest apical thickening of any of the species of the genus. In these respects the species form a series from large spores to small spores as follows: *P. philippinensis, P. clarkiana, P. mandschurica*. Since aeciospores have not been reported in *P. sydowii* it cannot be compared. Peridium-like cells adherent to the epidermis are prominent in *P. philippinensis*, occur in some collections of *P. clarkiana*, but have not been observed in *P. mandschurica*. Spermogonia are present in all three and are much alike. No dependable differences could be found in the primary or secondary telia. The secondary telia of *P. philippinensis* were found only in immature condition so that the length that the spore chains may attain is uncertain.

PUCCINIOSTELE HASHIOKAI (Hirat. f.) Cumm. (Mycologia **42**: 790. 1950) occurs on *Ampelopsis cantoniensis* in China and Formosa.

Spermogonia and aecia are unknown in this species. It forms uredia with peripheral paraphyses and echinulate spores and secondary telia. The discovery of the aecia will be necessary before the relationships of this species can be decided. While the telia and teliospores are generally similar to those of the species on *Astilbe* the phakopsoroid uredia are much different.

DISCUSSION

The relationships of *Pucciniostele* are obscure. Dietel (**2**) and the Sydows (**6**) place the genus in the Pucciniaceae with several other genera of dubious affinities. On the other hand Kursanov

et al. (**4**) and Hiratsuka (**3**) consider that the genus belongs in the Melampsoraceae. The latter treatment seems the more logical for several reasons: 1) peridiate uredia are common in the Melampsoraceae but very rare in the Pucciniaceae; 2) catenulate urediospores occur in the Melampsoraceae but not in the Pucciniaceae; and 3) catenulately produced teliospores adherent in chains occur in the Pucciniaceae only in the Pucciniosireae, a tribe of anomalous and mostly microcyclic genera. The lack of strong lateral adherence of the chains of teliospores is the only character present in *Pucciniostela* that would indicate relationship with the *Pucciniaceae*.

In the Melampsoraceae catenulate urediospores occur in *Mesopsora* (spermogonia and aecia unknown), in *Chrysomyxa* (aecia on *Picea*), and in *Coleosporium* (aecia on *Pinus*). The teliospores also occur in chains in *Chrysomyxa* and there is a very close similarity between the aeciospores and the urediospores of any one species, as pointed out by Saville (**5**), and this is also true of *Pucciniostele*. On the other hand, the aecia of *Chrysomyxa* are peridermioid and the teliospores have non-pigmented walls and germinate without a dormant period. The teliospores of *Pucciniostele* are only slightly pigmented but apparently are not germinable at maturity. Moreover, the spermogonia of *Chrysomyxa* are subepidermal. *Coleosporium* has subepidermal spermogonia and peridermioid aecia and, in addition, the teliospores produce internal basidia and are not typically catenulate. If *P. hashiokai* actually belongs in *Pucciniostele*, relationship to the *Phakopsora-Angiopsora* complex is possible. As mentioned above, this species has phakopsoroid uredia. Most species of *Angiopsora* also have such uredia and produce their teliospores in chains. Aecia have been reported only for *Phakopsora hansfordii* Cumm. (*Angiopsora hansfordii* Thirum. & Kern 1949, not *A. hansfordii* Cumm. 1945). In this species the spermogonia are similar in position and morphology to those of *Pucciniostele* but the aecia have a peridium. Moreover, in *Angiopsora* and *Phakopsora* the lateral adherence of the teliospores is stronger. *Phragmidiella* has similar telia, subcuticular but paraphysate spermogonia, uredinoid subcuticular aecia, and pedicellate urediospores.

While all of these genera have some points of similarity with *Pucciniostele*, all also have sufficient differences as to make it impossible accurately to determine the actual relationships. On the other hand, the evidence does suggest strongly that *Pucciniostele* belongs in the Melampsoraceae near the genera discussed.

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TWO COMMON LUMBER-STAINING FUNGI IN THE WESTERN UNITED STATES

ROSS W. DAVIDSON [1, 2]

(WITH 3 FIGURES)

In the summer of 1944 the writer obtained pure cultures of some staining fungi on coniferous lumber in Oregon and Washington. Time did not permit a thorough study of all the staining fungi encountered but it was found that there were two very common and conspicuous species. One of these, a common cause of dark stain on Douglas-fir sapwood, is very similar to the European fungus *Endoconidiophora coerulescens* Münch. The other species, on many coniferous woods, seems to be the same as the European species *Ophiostoma piceae* (Münch) Sydow. The present paper gives available information on these two fungi, neither of which is known to be present in the eastern or southeastern United States.

OPHIOSTOMA PICEAE

There is commonly present on Douglas-fir sapwood and on lumber of other western species, such as western hemlock, noble fir, and Sitka spruce, a fungus with a *Graphium* stage. It is usually found on wood having no pronounced stain, or it may be present on wood stained by other fungi.

When transferred in pure culture the *Graphium* or coremium stage forms very quickly (FIG. 1, E), along with conidia borne directly on the hyphae (FIG. 1, C). The cultures on malt agar medium may become dark gray or dark brown but often remain

[1] Senior Pathologist, Division of Forest Pathology, U. S. Dept. Agr., in cooperation with Rocky Mountain Forest Experiment Station and Colorado Agricultural Experiment Station. Colo. Agr. Expt. Sta. Scientific Paper No. 396.

[2] The writer was assisted by Ernest Wright, Division of Forest Pathology, U.S.D.A., in collecting much of the material from which cultures were obtained.

light gray to light brown. After several weeks long-beaked perithecia may begin to develop slowly in the cultures (FIG. 1, A). Usually perithecia are not abundant and are mixed in with the densely formed *Graphium* fruiting bodies. Some cultures do not form perithecia.

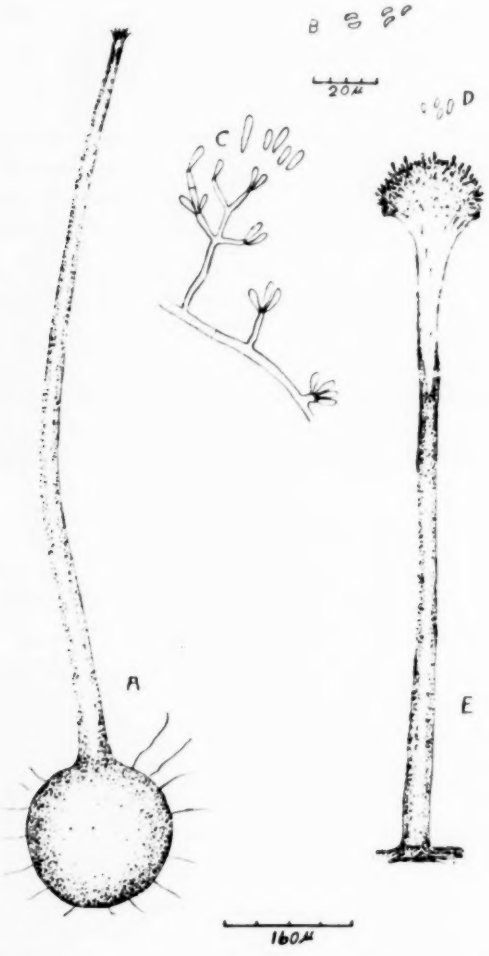

FIG. 1. *Ophiostoma piceae*. A. Perithecium; B. ascospores; C. conidia and conidiophores; D. conidia from *Graphium* stage sporophore; E. *Graphium* or coremium stage.

Morphologically this western fungus compares closely with that described as *Ophiostoma piceae*. Lagerberg *et al.* (**4**) state that this species develops very slowly and does not cause pronounced stain in coniferous wood. Growth rate in culture is about the same as that recorded for *O. piceae*.

The fungus is difficult to maintain in normal condition in culture on malt agar, a fact also pointed out by Lagerberg *et al.* (**4**). It grows well and forms abundant coremia when first isolated; but it changes when held in culture for a long period, first, by forming white to light brown sectors with few or no coremia or perithecia, and later, the entire culture may revert to a non-fruiting condition. This is also true for many of the wood-staining fungi, so that it is difficult to study and accurately identify any but fresh isolates. They probably could be maintained by frequent transfer and selection of only normal single-spore cultures each time stock cultures are transferred.

ENDOCONIDIOPHORA ON DOUGLAS-FIR

In a study of lumber-staining fungi on pine and hardwood lumber and logs in the Southeastern States in 1931 and 1932 (**2**), a very common species was found on hardwood. This species appeared morphologically similar to the European fungus *Endoconidiophora coerulescens* (**5**). It was later determined that the southern hardwood fungus was a distinct species and it was described as *E. virescens* (**3**). At that time it was pointed out that the European *E. coerulescens* was reported to occur only on pine and spruce, and it had a banana oil (amyl acetate) odor in culture besides showing slight morphological differences when cultures of it were compared with cultures of the southern hardwood fungus.

Infected sapwood of Douglas-fir lumber commonly shows a black surface growth and a gray staining of the wood below it. This stain is common on lumber in the central Rocky Mountain area, and probably in other areas. The lumber on which it has been observed was shipped from Oregon and infection is believed to have occurred in the woods or at the lumber mills. Infected lumber picked up on house construction jobs in Fort Collins, Colorado, harbored the fungus in viable condition (FIG. 2, A). While in Oregon in 1944, the writer visited a few Douglas-fir saw-

mills; he found the fungus at one mill in Portland and saw it in abundance on Douglas-fir slabs stacked along streets for fuelwood. Ernest Wright of the Division of Forest Pathology found it in check piles in stain control tests at Puyallup, Washington (FIG. 2, B).

The fungus mat on the surface of the wood consists of dense black mycelium and numerous black, hirsute perithecia. Where the mycelium is not too dense, the perithecia show up as small black tufts with a central neck or spine. Endoconidia are also present on the surface of infected wood but cannot be seen without

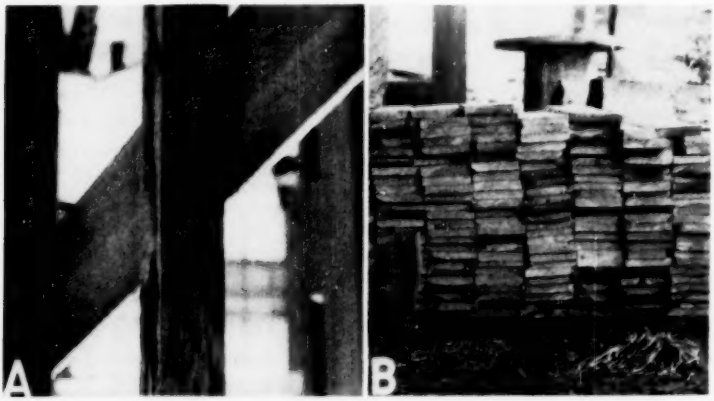

FIG. 2. *Endoconidiophora coerulescens* f. *douglasii* stain on Douglas-fir. A. On house framing timbers at Fort Collins, Colorado; B. on ends of untreated boards in stain control test pile at Puyallup, Washington. (B. was contributed by Ernest Wright.)

the aid of a good lens or dissecting microscope. The stain extends to the heartwood.

CULTURES OF ENDOCONIDIOPHORA. Success has been obtained in isolating the Douglas-fir staining fungus by collecting wood perithecia in good fruiting condition or by placing infected wood in a damp chamber until perithecia develop. Perithecia will usually develop on infected wood in 3 to 7 days after infection. When perithecia have matured, ascospores may be picked from the perithecial beaks with a sterile needle and transferred to cornmeal, malt, or potato dextrose agar media.

The fungus was obtained in pure culture in 1944 at Portland, Oregon. However, it did not grow well on the malt agar medium used and all cultures were dead on arrival at Beltsville, Maryland. The interval between the time of making the isolations and attempting transfers was 3 to 4 weeks. In 1945 the fungus was isolated at Beltsville from a short section of Douglas-fir "2 × 4" shipped from Portland, Oregon. These cultures also died after a short period, but during this time the fungus was compared with a culture of *Endoconidiophora coerulescens* from Europe. At a temperature of 22° to 25° C both fungi had the characteristic banana oil odor, but the European strain grew considerably faster than the Douglas-fir strain (47 mm. in 4 days and 81 mm. in 6 days for the European as compared to 11 mm. and 22 mm. for the American). The European culture would no longer produce perithecia and was somewhat faster growing than cultures described by Lagerberg *et al.* (**4**).

Later studies in a cooler laboratory at Fort Collins, Colorado, showed that the Douglas-fir fungus can be maintained satisfactorily in culture on any of the three culture media mentioned above. Growth has been much faster and more uniform than the earlier isolations indicated. It was not grown under constant temperature conditions. The temperature remained under 21° C and probably averaged between 16° to 18° C. Growth at this lower temperature was about the same as that recorded by Lagerberg *et al.* (**4**) for the European fungus, or about 100 mm. diameter in 10 days. Perithecia mature in culture in 7 to 12 days.

COMPARISON OF THE DOUGLAS-FIR FUNGUS WITH ENDOCONIDIOPHORA COERULESCENS. Morphologically, the *Endoconidiophora* on Douglas-fir is very similar to *E. coerulescens*. Perithecia are about the same in size, shape, length of neck, and in character of ostiole. Ascospores are 6–9 × 1.7–2 μ, which is about the same as given for the European fungus. Conidiophores are of two types, one tapering to a small diameter at the apex and the other enlarged to the apex. This character was not noticed in cultures of *E. coerulescens* from Europe, although conidia had about the same variability in size in both, with those from Douglas-fir ranging slightly smaller in diameter.

The hosts of these two fungi are different. In the United States the fungus has been found only on Douglas-fir. It has not been observed on spruce, hemlock, Sitka spruce, true firs, or on pine species in any areas where the writer has made observations. However, a sufficiently thorough search has not been made in some areas of the West to be sure it does not occur on some other host. On the basis of present information, it seems desirable to designate the western fungus a form of *E. coerulescens:*

Endoconidiophora coerulescens Münch forma **douglasii** f. nov.[3] (FIG. 3).

Perithecia atra, hirsuta, 160–190 μ lata, 180–210 μ alta, rostellis basi 30–40, apice 12–16 μ latis, 500–1000 μ longis, filamentis hyalinis 15–24 μ longis, basi 2 μ, ad apicem ad 1 μ attenuatis praeditis; ascosporae subcurvatae, hyalinae, 6–9 × 1.7–2 μ; endoconidiophora 50–250 μ longa, 5–10 μ crassa, alia apice attenuata, alia supra inflata; conidia 4–30 × 3–8 μ, hyalina, catenulata.

In ligno *Pseudotsugae taxifoliae* U. S.

Perithecia black, hirsute, 160–190 × 180–210 μ high, with necks 30–40 μ wide at base to 12–16 μ wide at tip and 500–1000 μ long, ostiolar filaments hyaline, 15–25 μ long, and tapering from about 2 μ at the base to 1 μ at tip; ascospores slightly curved, hyaline, 6–9 × 1.7–2 μ; endoconidiophores variable in length and thickness, 50–250 μ long by 5–10 μ thick, some tapering to smaller diameter at apex and others enlarged above; conidia variable in length and thickness, 4–30 × 3–8 μ, hyaline, in chains. Grows on cornmeal, malt, or potato dextrose agar media at temperatures slightly lower than ordinary room temperature of 25° C.

Collected on sapwood of *Pseudotsuga taxifolia* (Poir.) Britton in the western United States.

Type specimen (F. P. *70703*) deposited in the Mycological Collections, Bureau of Plant Industry, Beltsville, Md.

SINGLE-SPORE CULTURES. Twenty-three of 33 single conidial and single ascospore cultures developed fertile perithecia. Ten of the single-spore cultures developed no perithecia, either when grown separately or when grown together in all possible combinations. All ten nonperithecial cultures had perithecial primordia. Bakshi (**1**), who isolated *E. coerulescens* from pine wood in Eng-

[3] Latin diagnosis prepared by Edith K. Cash, Associate Mycologist, Division of Mycology and Disease Survey, Bureau of Plant Industry, Soils, and Agricultural Engineering, Beltsville, Maryland.

land, considered the species to be homothallic but some of his single-spore cultures did not develop perithecia.

An attempt was made to spermatize the 10 non-perithecial cultures by streaking spores and mycelium from a single-spore perithecial culture across 5-day-old petri-dish cultures. Fertile perithecia developed at or near the margins of the 5-day-old growth

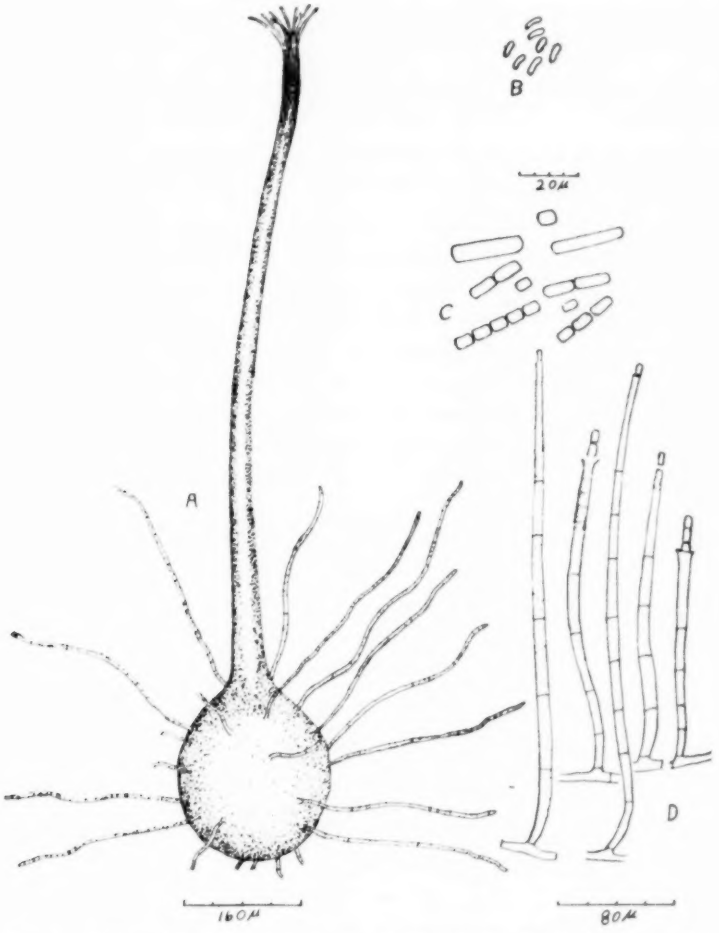

FIG. 3. *Endoconidiophora coerulescens* f. *douglasii*. A. Perithecium; B. ascospores; C. conidia; D. endoconidiophores with condidia.

in all 10 cultures in 6 days. These perithecia developed only in the area of the streaks. Cultures from fertile (single-spore) strains started at the same time required 8 days for the development of mature perithecia. This would seem to indicate either that some of the perithecial primordia that were in the proper stage of development were spermatized by the spores or mycelium from a normal perithecial culture, or that some combination of the two stimulated development of perithecia of the fertile strain more quickly than occurs under normal conditions.

SUMMARY

Two species of the *Ophiostomataceae* group of wood-staining fungi are reported on sapwood of coniferous lumber in the western United States. A species very similar to *Endoconidiophora coerulescens* but apparently confined to Douglas-fir is described as a form of the European species. It covers the sapwood of Douglas-fir with a black surface growth of mycelium, and stains the wood likht to dark gray. *Ophiostoma piceae*, another well-known European species, is also common on Douglas-fir lumber and numerous other coniferous woods in the West. It does not cause a pronounced stain but its *Graphium* fruiting stage is conspicuous on the wood.

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ON HYALODOTHIS CARICIS

WILLIAM W. DIEHL

(WITH 4 FIGURES AND MAP)

Among the many puzzling examples of immature and generally unrecognizable fungi that for some thirty years have come to my hand for determination there is a small number of specimens containing inflorescences of various species of *Carex* bearing similar, immature stromata that at each encounter I have hesitantly labeled "*Hyalodothis* (?) *caricis* Pat. & Har." because no other name seemed to apply. It is always embarrassing to assign a name to an immature fungus, and in these cases especially so because they were mostly found at points many thousands of miles apart along the northern and western periphery of the Pacific Ocean.

The species was questioningly described from immature stromata by Patouillard and Hariot in 1893 (Bull. Soc. Myc. Fr. **9**: 210–211) and is known only from their record of the two specimens found in 1889 by the Abbé Urbain Faurie on the Japanese Island of Kunashiri. The type material (FIG. 1) among Patouillard's specimens at the Farlow Herbarium of Harvard University, examined through the courtesy of the late Dr. David Linder, was found to resemble closely the specimens that are thought to be conspecific with it. Insofar as immature stromata are significant, they could all be representative of the same species. The black subspherical stromata, which suggest small shoe-buttons, arise upon immature florets and in some cases entirely enclose them. They show no hypothallus and, since their superficial conformation and habit suggest a member of the Balansieae, they might be referable to *Balansiopsis* were there characteristic clavicipitaceous perithecial walls in evidence. This lack of a hypothallus should serve to preclude confusing this species with the cariciphilous Indian *Ophiodothis caricis* Berk. & Curt. because, as shown by Atkinson (Jour. Mycol. **11**: 248–267. 1905), that species pos-

sesses a hypothallus, which he termed the "sclerotium." But without more matured frutifications to interpret its taxonomic relations *H. caricis* remains a taxonomic enigma.

There appears to be no subsequent report of the species unless, as seems likely, the two inadequate records of *Balansia vorax* by K. Togashi (Japan Jour. Bot. **2**: 80. 1924) on *C. sachalinensis* from the Island of Rebun and by K. Togashi and F. Onuma (Bull. Imp. Coll. Agr. & For. Morioka **17**: 10. 1934) on *C. morrowi* from Iwate Province in northern Japan may refer to it. No Japanese specimens other than Faurie's have been available for examination but the comparable specimens now available from other Pacific areas are as follows:

In the Farlow Herbarium there is a fragment of an immature specimen labeled "*Ophiodothis vorax* on *Carex buxbaumii*" found at Sitka, Alaska in 1867 by J. T. Rothrock which had been turned over to W. G. Farlow from the Gray Herbarium. This specimen seems to have escaped any published record although Rothrock in his "Sketch of the Flora of Alaska" (Rept. Smithsonian Inst. **1867**: 57. 1868 [1872]) records *C. buxbaumii* from Sitka.

At Sitka J. P. Anderson in 1916 also found similar but smaller stromata on a different sedge, *C. pauciflora*.

In 1939 W. J. Eyerdam at Knight Island on Prince William Sound in Alaska obtained analogous immature stromata in two different gatherings of *C. limosa*. But in these instances the stromata, instead of being few and occasional on the inflorescences, were so numerous and so strikingly aggregated as to give each inflorescence of the host the appearance of a small blackberry (FIGS. 2, 3). A comparable blackberry-like mass of stromata is additionally available on a single spike of *C. enanderi* found in 1945 on the Island of Attu by G. W. Soule.

In 1943 Capt. G. A. Ammann on the Island of Kiska made two gatherings of similar immatured stromata which are possibly less crowded (FIG. 4), thus more like the type, among the florets of

FIGS. 1–4. *Hyalodothis caricis*. 1. Type material, × approx. 1, at Farlow Herbarium. Photo courtesy David Lindner. 2. On *C. limosa* W. J. Eyerdam no. *3232*, × 1. Photo by R. L. Taylor. 3. On *C. limosa*, × ½, showing habit. Photo by R. L. Taylor. 4. On *C. lyngbyei*, G. A. Ammann, × 1. Photo by R. L. Taylor.

FIGS. 1–4.

TABLE SUMMARIZING RECORDS OF *H. caricis* INDICATED ON MAP

Station	Lat.	Host	Date	Collector	Previous record in literature	Herbaria where deposited	Host determined or verified by:
+Japan-Isl. Kunashiri	48° N	*Carex* sp.	1889	U. Faurie	Patouillard & Hariot 1889	Farlow Herbarium	U. Faurie—?
−Japan-Isl. Rebun	40° N	*C. sachalinensis* Kuek.	1922	T. Togashi No. 18	Togashi 1924	?	Togashi—?
−Japan-Pref. Iwate	40° N	*C. morrowi* Boott	1928	F. Onuma No. 29	Togashi & Onuma 1934	?	Togashi & Onuma —?
+Alaska-[Sitka?]	57° N	*C. buxbaumii* Wahl	1867	J. T. Rothrock	0	Farlow Herbarium	Asa Gray—?
+Alaska-Sitka	57° N	*C. pauciflora* Lightf.	1916	J. P. Anderson No. 348	0	U. S. Myc. Collections, Beltsville, Md.	J. P. Anderson—?
+Alaska-Knight Isl.	61° N	*C. limosa* L.	1939	W. J. Eyerdam	0	U. S. Myc. Collections, Beltsville, Md.	F. J. Hermann
+Alaska-Kiska	52° N	*C. lyngbyei* Hornem.	1943	G. A. Ammann Nos. 20, 111	0	do & Herb. Univ., Mich.	F. J. Hermann
+Alaska-Attu	53° N	*C. enanderi* Hulten	1945	G. W. Soule No. 541	0	U. S. Myc. Collections, Beltsville, Md.	F. J. Hermann
+New Guinea	7° S	*C.* sp.	1939	M. S. Clemens No. 9567	0	U. S. Myc. Collections, Beltsville, Md.	F. J. Hermann
+New Guinea	7° S	*C. rara* Boott	1939	M. S. Clemens no No.	0	U. S. Myc. Collections, Beltsville, Md.	F. J. Hermann

+ = specimens examined; − = specimens not seen.

C. lyngbyei. Ammann's specimens have been made available through the courtesy of Dr. E. B. Mains of the University of Michigan.

Two specimens from New Guinea found by Mrs. M. S. Clemens in 1939 at altitudes between 7000 and 10,000 feet are similar although they are slightly more mature than the northern specimens noted above; they bear immature asci in locules without as yet the development of the perithecial walls characteristic of the Clavicipitaceae; one of the New Guinea specimens is on an undetermined species of *Carex*, the other on *C. rara*.

Although the blackberry-like masses of stromata in three of the above-cited specimens present a superficial aspect very slightly different from the others the characters of the several stromata are not significantly different. The high degree of aggregation in these instances may merely be indicative of a differental susceptibility reaction in these particular hosts rather than of a taxonomic difference in the fungus. The stromata are easily broken from their points of attachment and if subjected to the pelting of rain or snow in the winter season would doubtless be detached and fall to the ground. The peculiar and consistently immature state of the stromata, according to the seasonal lateness of dates of collection of the northern specimens, would have precluded their further maturity during the current season. It would thus seem that matured stromata would be found during the second season upon the ground rather than attached to inflorescences. The same effect could likewise be expected for those stromata produced at higher elevations farther to the south.

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A NEW SPECIES OF MONOBLEPHARELLA [1]

(WITH 1 FIGURE)

FREDERICK K. SPARROW [2]

The genus *Monoblepharella,* consisting of four species, is a small but interesting group of Phycomycetes by reason of the fact that the fertilized egg is propelled by the flagellum of the male gamete (Sparrow **1940**; Shanor **1942**; Springer **1945**). It has assumed further interest with the discovery by Johns (**1952**) that members of the well-known genus *Gonapodya* possess an essentially similar type of sexual reproduction.

The purpose of the present paper is to report a species of *Monoblepharella* which is distinct in certain essential features from previously described ones, namely, in the apparent lack of antheridia (and antherozoids), and in the formation of apogamous oospores which remain within the oogonium. It was isolated from soil collected in a roadside ditch in the immediate vicinity of Clarksdale, Mississippi, February 16, 1949.

The mycelium was of the delicate, flexuous, pearly gray type described for other species of *Monoblepharella.* Although most species of the genus possess irregularities of the mycelium, the present fungus was noteworthy in this respect. Numerous, up to 9 μ in diameter, catenulate expansions of the hyphae were formed which in some instances strikingly resembled those found in *Gonapodya.* Further resemblance to the latter genus was found in the frequent emergence from the distal catenulation of two diverging hyphal elements. The contents of the hyphae were disposed in the usual reticulate fashion save in actively growing apices which were homogeneous.

Zoosporangia were abundantly formed at about 24.5° C. The precise temperature relationship has not, however, as yet been

[1] Paper no. 978, Department of Botany, University of Michigan.

[2] Acknowledgment is made to the Executive Board of the Horace H. Rackham School of Graduate Studies for a grant which made possible the collection of this and other fungi in the Gulf States.

ascertained. These sporangia were of the usual siliquaeform type and were 34–47 μ long by 5–8 μ in diameter. They were borne either singly or in pairs at the tips of hyphae. After sympodial branching of the hyphae they appeared lateral, often in clusters (FIG. 1, a, b). The zoospores were ovoid to subcylindrical when swimming, 8 μ long by 5 μ wide, and possessed the usual monoblepharid internal structure and posterior flagellum (FIG. 1, b).

Oogonia were abundantly formed when water cultures were left undisturbed for several days at 30–31° C. They were obpyriform with rounded apex, narrow cylindrical base, and were 10–15 μ long by 7.5–8.75 μ in diameter, tapering to 2.5 μ at the base. Like other species of the genus, they contained in most instances (FIG. 1, e, compared with FIG. 1, f) a single egg bearing numerous large refractive globules. These oogonia were at first terminal (FIG. 1, g) but by sympodial branching of the hyphae often become lateral and occasionally clustered (FIG. 1, c, d, f, h).

No evidence has been found as yet for the existence of antheridia and antherozoids. This feature has been considered in some detail. Pure cultures on agar were obtained from which transfers were made to sterile soil water cultures baited with hempseed. Under these conditions an abundance of oogonia (and oospores) were formed, but so far as could be ascertained, no antheridia or other reproductive structures of any kind. Needless to say, this aspect of the fungus is still under investigation.

Under the aforementioned conditions oospores matured in profusion and these were uniformly endogenous. The latter fact was in agreement with observations in the original gross cultures and seemed to indicate that, typically, the oospores were endogenous and not so because of fouled environmental circumstances. Further work on the morphology and studies of the physiology of the fungus are being undertaken.

Since by reason of the aforementioned features the fungus is very distinct from other species of *Monoblepharella*, in which genus it undoubtedly belongs, it is described as new.

Monoblepharella endogena n. sp.

Mycelium amplum, hyphis tenuibus, flexuosis, ramosis, 2.5–6 μ dia. intus reticulate vacuolatis, plerumque multis irregularibus tumoribus ad 9 μ dia.

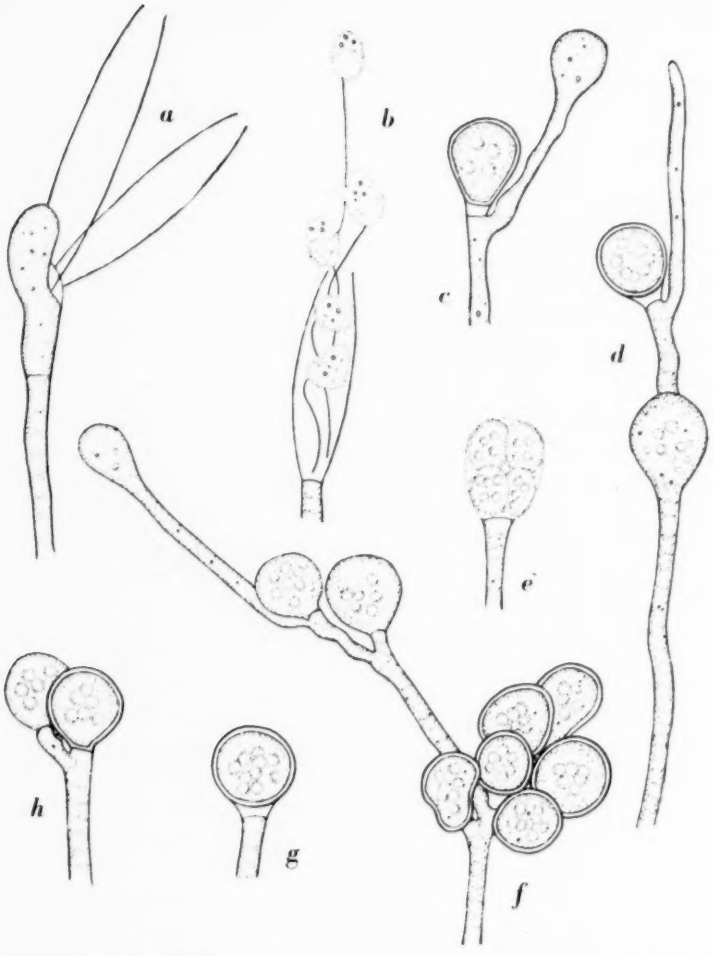

FIG. 1, a–h. *Monoblepharella endogena*. All ×970. a. Cluster of sporangia, two empty, a third developing. b. Zoosporangium discharging its zoospores. c, d. Mature endogenous oospores on hyphae developing new oogonia. e. Oogonium with four eggs. f. Cluster of mature oospores with new oogonia forming sympodially. g. Terminal oospore. h. Terminal oospore which by sympodial branching and subsequent oogonial formation of its supporting hypha appears lateral.

Sporangia siliquiformia, 34–47 μ longa, 5–8 μ dia., apices hypharum terminantia vel singula vel bina vel post hyphae ramificationem sympodialem quasilateralia. Zoosporis ovoideis vel subcylindricis, 8 μ longis, 5 μ latis postice cilio quam soma duplo vel triplo longiori praeditis. Oogonium primum terminale vel post ramificationem hyphae sympodialem saepe quasilaterale, clavatum vel obpyriforme, apice rotundatum basi anguste cylindricum, 10–15 μ longum, 7.5–8.75 μ crassum basi ad diametrum 2.5 μ angustatum, maturitate ovo singulo (rare ad quatuor) magno, refractivis globulis praedito, incluso. Antheridium ignotum. Oospora endogena, spherica, 7.5–10 μ dia., etiamque guttulata membrana paulum incrassata pallide brunnea laevia.

In humo, per *Cannabis* semen illecebram culta, coll. F. K. Sparrow, Clarksdale, Mississippi, Feb. 16, 1949.

Mycelium well developed, of tenuous flexuous branched hyphae 2.5–6 μ in diameter, with many irregular swellings up to 9 μ in diameter, contents reticulately vacuolate. Sporangia siliquaeform, 34–47 μ long by 5–8 μ in diameter, occurring singly or in pairs at tips of the hyphae or after sympodial branching appearing lateral; zoospores ovoid or subcylindrical, 8 μ long by 5 μ wide, the posterior flagellum 2–3 times the length of the body. Oogonium at first terminal or after sympodial branching of hypha appearing lateral; clavate or obpyriform with rounded apex and narrow cylindrical base, 10–15 μ long by 7.5–8.75 μ wide, tapering to 2.5 μ at base, the contents at maturity forming one, rarely up to 4 eggs bearing numerous large refractive globules. Antheridium and antherozoids unknown. Oospore spherical, 7.5–10 μ in diameter, endogenous, with a slightly thickened light-brown smooth wall, contents bearing globules.

In soil, cultivated on hempseed. Clarksdale, Mississippi, collected February 16, 1949, F. K. Sparrow.

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SOIL AS A STORAGE MEDIUM FOR FUNGI [1]

ALEXANDER BAKERSPIGEL

(WITH 1 FIGURE)

Since 1918 a number of workers (**3**, **15**, **16**) have stored fungi for months or even years upon sterilized soil. Thus, in 1934 Green and Fred (**10**) preserved *Penicillium chrysogenum* Thom. and *Aspergillus* ssp. for over 2 years in soil. They found that after this time the fungi were viable and their colony characters were less changed than the control cultures growing on nutrient agars. Similarly from 1945–52, Miller (**11**, **12**, **13**), Miller *et al.* (**14**), Cormack (**6**) and Gordon (**9**) found soil to have a preservative and stabilizing effect upon Fusarium ssp.

In 1948, Ciferri and Redaelli (**5**) inoculated 3 species of dermatophytes on soil and preserved them for 3 years observing little pleomorphic change.

Dowding (**7**) and Emmons (**8**) have grown systemic fungi on soil. In 1951, Ajello and Zeidberg (**1**) and Emmons (**8**) isolated *Histoplasma capsulatum* Darling and *Allescheria Boydii* Shear from soil in nature. However as far as the writer is aware the systemic fungi have not been stored in the laboratory in this medium.

The following investigation, as well as determining the viability of additional species of fungi upon soil, compares the advantages of loam with clay, peat, sand and other natural materials, records whether or not changes in colony characters occur during storage, and measures the amount of increase of the inoculum.

[1] Contribution from the Department of Botany, McMaster University, and the Provincial Laboratory of Public Health, University of Alberta.

MATERIALS AND METHODS

MATERIALS. 18 fungi were used for the storage experiments. They were members of the Fungi Imperfecti, chiefly plant and animal pathogens. The species and their sources are as follows.

Group A

1. *Phoma* sp. from chrysanthemum, Ontario; 2. *Hormodendrum* sp.*a* soil, Ontario; 3. *Fusarium* sp., muskmelon, Ontario. 4. *Hormodendrum* sp.*b*, *Dracaena*, Ontario; 5. *Phyllosticta* sp., *Dracaena*, Ontario.

Group B

Dermatophytes: 1. *Trichophyton mentagrophytes* (Robin) Blanchard from patient 1453, Alberta; 2. *T. tonsurans* Malmsten and 3. *T. sulfureum* Sabouraud, both from patients in Ontario; 4. *Epidermophyton floccosum* (Harz) Langeron and Milochevitch, from patient 2053, Alberta; 5. *Microsporum audouini* Gruby, patient 2792, Alberta; 6. *M. canis* Bodin, patient 2794, Alberta; 7. *M. gypseum* (Bodin) Guiart and Grigorakis, patient, Ontario.

Systemic Fungi: 1. *Candida albicans* (Robin) Berkhout from patient 1891, Alberta; 2. *Sporotrichum Schenkii* (Hektoen and Perkins) Matruchot, patient, Michigan; 3. *Blastomyces dermatitidis* Gilchrist and Stokes, dog, Iowa; 4. *Histoplasma capsulatum* Darling, the U. S. Department of Health Culture Collection; 5. *Haplosporangium parvum* Emmons, deermouse lung, Alberta; 6. *Nocardia asteroides* (Eppinger) Blanchard, a patient, Michigan.

The storage materials for group A were loam *a*, sand, clay, peat and other natural materials whose origin and physical properties are shown in TABLE I.

The sole storage material for group B was loam *b*.

INOCULATION PROCEDURE. For group A, 135 cc. of the 7 storage materials (listed in TABLES II and III) were distributed into duplicate sets of 250 cc. Ehrlenmeyer flasks. For group B, 135 cc. of loam *b* alone were distributed into flasks. Distilled water equal to 25% of the water-holding capacities of the materials used was poured into each flask. The flasks were plugged with non-absorbent cotton and then autoclaved once at 15 lbs. pressure for

TABLE I

STORAGE MATERIALS AND SOME OF THEIR PHYSICAL PROPERTIES

Materials	Source	Weight of 135 cc. oven-dried material in gms.	Water holding capacity per 100 cc.	Density	pH
Loam *a*	Royal Botanical Gardens, Ontario	121.5	32	0.90	6.8
Loam *b*	Plant Science Dep't, University of Alberta	154.0	47	1.25	6.1
Clay	McMaster University, Ontario	183.5	22	1.36	7.0
Oak Sawdust	McMaster University, Ontario	15.5	35	0.12	6.9
Humified Peat	McMaster University, Ontario	36.5	94	0.27	6.5
Unhumified Peat	McMaster University, Ontario	9.5	191	0.07	6.7
Banding Sand	Central Scientific Co., Ontario	236.5	31	1.75	6.8
Kieselguhr	Peller Brewing Co., Ontario	16.2	115	0.12	7.2

20 minutes, which proved sufficient to prevent the growth of contaminants.

The 18 fungi were grown on agar slants from 1 to 3 weeks. Sufficient sterilized distilled water was added to obtain conidial suspensions. These were diluted to reach volumes equal to 25% of the water-holding capacity of the storage material to which they were added.

TABLE II

AVERAGE NUMBER OF VIABLE CELLS PER CC. STORAGE MATERIAL *

Fungus	Storage material	Age of sample (in months)									
		5	10	13	15	18	20	23	30	39	48
Phoma	Loam *a*	4900	4700	7800	5400	6500	7300	2000	2000	100	30
	Clay	6400	3000	2300	3300	2300	1500	0			
	Sawdust	600	100	0							
	Humified Peat	2800	400	900	100	800	0				
	Unhumified Peat	1700	100	100	0						
	Sand	800	0								
	Kieselguhr	1400	200	200	100	0					
Hormodendrum *a*	Loam *a*	2200	0								
	Clay	1100	0								
	Sawdust	0									
	Humified Peat	0									
	Unhumified Peat	0									
	Sand	100	0								
	Kieselguhr	300	0								
Fusarium	Loam *a*	7200	5000	7100	8400	8700	25000	10300	5000	100	80
	Clay	1500	300	400	900	1500	1000	0			
	Sawdust	6000	200	100	300	0					
	Humified Peat	2200	8400	8500	8800	8000	9000	2800	1500	1000	50
	Unhumified Peat	1300	2600	300	1200	1300	1000	0			
	Sand	100	0								
	Kieselguhr	2300	200	0							

* Approximate number of viable cells inoculated per cc. material was: for *Phoma* 104, *Hormodendrum a* 48 and *Fusarium* 44.

TABLE III

AVERAGE NUMBER OF VIABLE CELLS PER CC. STORAGE MATERIAL *

Fungus	Storage material	Age of sample (in months)							
		1	2	3	4	7	14	23	32
Phyllosticta	Loam *a*	132,000	375,000	520,000	600,000	290,000	450,000	60,000	85,000
	Clay	54,000	75,000	166,000	178,000	40,000	23,000	4,000	11,000
	Sawdust	5,000	39,000	36,000	17,000	12,000	9,000	2,200	1,000
	Humified Peat	58,000	27,000	43,000	22,000	7,000	0		
	Unhumified Peat	54,000	112,000	92,000	44,000	11,000	0		
	Sand	11,000	49,000	47,000	8,000	2,000	0		
	Kieselguhr	14,000	10,000	38,000	66,000	90,000	130,000	54,000	80,000
Hormodendrum b	Loam *a*	290,000	536,000	2,300,000	2,250,000	2,100,000	450,000	15,000	12,000
	Clay	256,000	326,000	1,100,000	965,000	995,000	200,000	53,000	1,000
	Sawdust	113,000	204,000	455,000	420,000	0			
	Humified Peat	361,000	154,000	100,000	200,000	0			
	Unhumified Peat	21,000	45,000	32,000	220,000	0			
	Sand	132,000	118,000	106,000	3,000	0			
	Kieselguhr	168,000	132,000	310,000	840,000	flask broken			

* Approximate number of viable cells inoculated per cc. material was: for *Phyllosticta* 1333 and *Hormodendrum b* 1207

As controls, the fungi were inoculated to fresh agar slants, group A on potato-dextrose-agar (Difco), group B on Sabouraud's medium in which phytone was substituted for peptone and yeast extract added. All of them were transferred to fresh agar each time the storage flasks were sampled.

The flasks containing the fungi of group A were stored at room temperature from 3 to 4 years, those containing group B, over a year.

In group A, when the flasks were about to be inoculated, the number of viable cells to be introduced was determined by the plate dilution method. For group B, no counts were made.

SAMPLING PROCEDURE. Group A: To test a sample, 10 cc. of storage material was measured from each flask into a sterilized graduated aluminum cup. It was transferred to the first of a dilution series of water blanks. The first blank was shaken 3–5 times to break up the material and then dispensed to the other blanks in the series, distributing the material by drawing it up and down several times with a sterile pipette. One milliliter of the final dilutions of the fungi was delivered to each of 6 plates. Potato-dextrose-agar was then added and the agar and inoculum rotated gently. Incubation was at 25° C for 5 to 10 days.

Group B: To test a sample, a small quantity of loam was removed by a sterilized platinum loop and streaked on the agar. Incubation was at 24° C for 1 to 3 weeks.

As can be seen from TABLES II and III, in group A, *Phoma* sp. and *Fusarium* sp. were sampled 10 times in a period of 4 years while *Hormodendrum* sp. *b*, and *Phyllosticta* sp. were sampled 8 times in a period of 2½ years. In group B, all the dermatophytes and systemic fungi were sampled 4 times during the year of storage.

TABLES II and III record the average number of colonies that developed on the plates.

CONDITION OF STORED FUNGI

VIABILITY. The results of the storage of group A upon 7 materials (TABLES II and III) show that the fungi remained viable from 1 to 4 years.

Loam is the most satisfactory material for storage. It is true

that one of the two Hormodendra eventually died upon it, but the large number of viable cells counted at the end of 5 months showed loam to be the most satisfactory medium for this fungus also. The two fungi that survived storage for 48 months (*Phoma* sp., *Fusarium* sp.) did so on loam.

Since loam *a* proved to be the best medium for group A, the 13 fungi of group B were all stored on loam. The source of the loam used for them (loam *b*) is shown in TABLE I. At the time of writing (13 months later) all these dermatophytes and systemic fungi are still viable on loam. In this respect therefore, loam *b* was superior to Sabouraud's agar on which one dermatophyte, *Epidermophyton floccosum*, did not survive longer than 3 months.

STABILITY. Of the 18 fungi growing on control agar-slants, a number of species became pleomorphic. Thus, in group A, *Fusarium* produced great amounts of white hyphae which overgrew the original pink mycelium while *Phyllosticta* and *Phoma* produced at the center of the colony tufts of brownish aerial mycelium which extended peripherally. In group B, every one of the dermatophytes produced white tufts of aerial mycelium which overgrew the cultures so that they became indistinguishable one from another.

On the other hand when cultures were made from flasks of loam *a* and loam *b* on which the above fungi had been stored, the new mycelia were in the "wild" or "primary" state with no trace of pleomorphic growth on any of them.

FIG. 1 *a, c, e* shows in the left hand vertical column wild type cultures of *T. mentagrophytes, M. gypseum* and *Phoma* sp. These were obtained from loam *a* and *b* on which they had been stored from 13 to 48 months. The right hand vertical column shows in *b, d* and *f* the pleomorphic characters of these fungi after the same period of storage on the control agar slants.

INCREASE. In the first 1–2 months following inoculation, the storage material was moist and was covered and permeated by webs of mycelium and masses of spores. Later the soil dried up and showed no visible trace of fungus.

In *Phoma, Fusarium, Hormodendrum b,* and *Phyllosticta,* during the storage period, the viable cells per cc. of loam increased

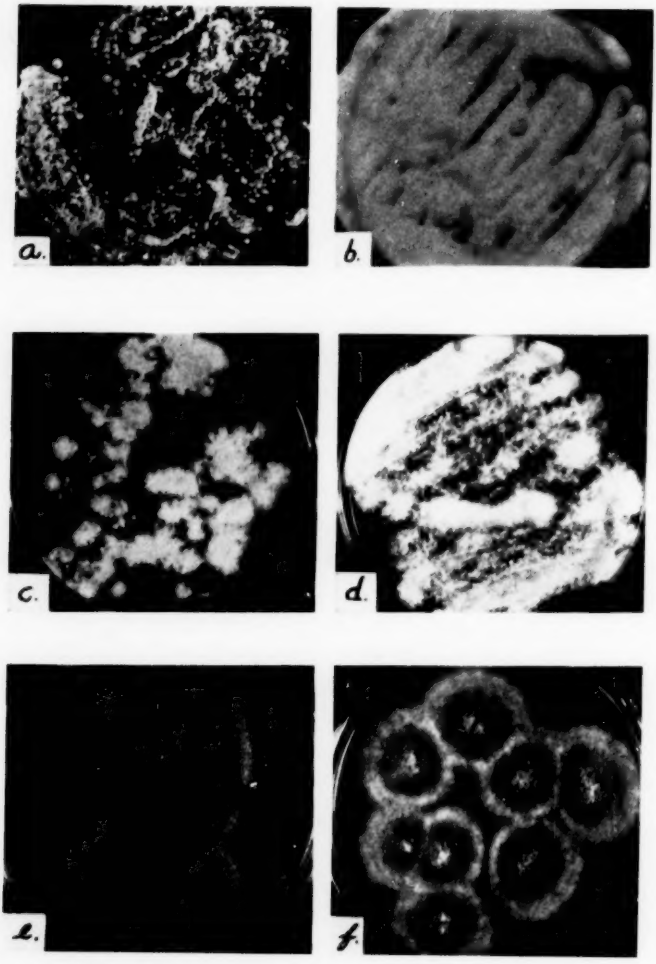

FIG. 1. *Wild* and *pleomorphic* cultures of fungi. Left, fungi after storage from 13 to 48 months on loam *a* and *b*. Right, controls maintained for the same time on agar. *a* and *b*, *Trichophyton mentagrophytes*; *c* and *d*, *Microsporum gypseum*; *e*, and *f*, *Phoma* sp. *a* and *b*, 12 day old cultures on Sabouraud's dextrose agar; *c*, 9 day; *d*, 11 day; *e* and *f*, 8 day. The cultures *b*, *d* and *f* are either completely pleomorphic or show centres and peripheries with tufts of mycelium. All photographs ½ natural size.

from 75 to 2000 fold. This increase went on for even 20 months after the material had become dry.

Explanations for this unexpected occurrence might be that, in the initial moist phase, clumps or chains of conidia were formed, then during prolonged storage, these clumps and chains of conidia (and mycelial strands also) broke up, causing an increase in the number of viable cells. The mixing of the contents of the flasks prior to sampling aided in this process. *Erysiphe polygoni* and *Piricularia oryzae* (**4**, **17**, **18**) are able to germinate under low relative humidities. Perhaps additional spores of our species of fungi germinated as the soil dried out, causing an increase in the number of viable cells.

ACKNOWLEDGMENTS

I am grateful to Dr. J. J. Miller, McMaster University for his direction during the earlier stage of this work and to Dr. E. S. Dowding, University of Alberta for her kind assistance during its later stage.

SUMMARY

1. Of 18 species of fungi stored on loam, 17 remain viable for 1 year, and 2 for as long as 4 years.

2. All the fungi that were preserved in soil remained in the original wild or primary state, whereas on agar the dermatophytes, *Phoma* sp., *Phyllosticta* sp. and *Fusarium* sp. became pleomorphic.

3. In loam, the cells of *Phoma*, *Fusarium*, *Phyllosticta* and *Hormodendrum b* increased throughout the medium.

4. This investigation suggests that soil storage of fungi in the laboratory would obviate the laborious periodic transfer of agar stock-cultures and would provide a rich source that may be drawn from repeatedly for years. Most important, it would prevent pleomorphic changes in the fungi.

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W. LAWRENCE WHITE

R. G. H. Siu and E. T. Reese

(WITH PORTRAIT)

Doctor W. Lawrence White, Director of the Farlow Library and Herbarium and Associate Professor of Botany, Harvard University, died on July 30, 1952 at the age of 44. With his passing the mycological world lost one of its leading and potentially dominant taxonomists and stimulating personalities. Recognized among mycologists for his pioneering work in several areas of taxonomic mycology he was known even more widely for his reputation as an authority on Tropical Deterioration by the military, industrial and scientific world. With his appointment in 1948 as the new Director of the Farlow Herbarium he soon renovated the organization which had lain fallow since the passing of Dr. David Linder. Clearly a renaissance was in progress. Prospects for the maturation and harvest of his own research efforts and for the future of the Farlow could not have been brighter. But the anticipation was not to be fulfilled. All was obliterated in a split-second by a dreadful automobile accident on Concord Pike on rainy July 27, 1952.

Dr. White was born in Salina, Pennsylvania, on May 29, 1908. His undergraduate days were spent at the Pennsylvania State College. There he was deeply influenced by Professor L. O. Overholts, whom he served as laboratory assistant. They had much in common—farm background, modesty, friendliness, sincerity, ability and, above all, an intense devotion to taxonomy. A strong bond of affection naturally developed. Many of the professor's mannerisms and work habits were assiduously copied by the young student. One of these, which was to serve Dr. White well later in life, was the almost fanatical accumulation of mycological facts and tidbits for future reference. Under the steady guidance of Professor Overholts, White gained an early intimacy

with the Basidiomycetes both in the field and the laboratory. His knowledge of the Polyporaceae and the Thelephoraceae was supplemented by the acquisition of a good understanding of the rusts from Professors F. D. Kern and H. W. Thurston, and of the smuts from Professor G. L. Zundel. It was unusual that an undergraduate was provided such excellent and intensive mycological guidance. Even more important to White was the friendly interest shown him by these professors, and by Dr. J. W. Sinden. The latter was largely responsible for his going to Cornell after receiving the baccalaureate degree in 1934.

A different directional influence was exerted during his graduate study at Cornell University. Laboratory emphasis replaced field trips. From his major professor, Dr. H. M. Fitzpatrick, he learned to channel his propensity for collecting tidbits into logical order and coherent taxonomic patterns. Through Professor H. H. Whetzel he developed an enthusiastic interest in the inoperculate discomycetes. For his own specialization Dr. White selected the large and complex group of the Helotiaceae. He plunged into the examination of the group with such skill and intensity that within five years he was thoroughly familiar with these fungi. Unfortunately, economic pressure forced Dr. White to accept more remunerative employment as soon as he completed his doctorate in 1940. While several papers covering small segments of this research did reach publication, a monograph was never put into final shape. Its completion would have been a significant contribution to mycology; and he was the one best qualified to execute it. During the later years, he frequently talked about the manuscript with deep-felt nostalgia. There was, at times, a suggestive twinkle in his eyes—possibly some of his future graduate students would complete the work he had started. Had fate been generous with the years this might have come to pass. His graduate years were interspersed with seasonal employment, which rounded out his mycological training. Two summers were spent studying Ascomycetes with Dr. F. J. Seaver at the New York Botanical Garden (1936, 1937). Two more were spent working on the Dutch Elm disease.

Having familiarized himself with Basidiomycetes and Ascomy-

cetes, Dr. White selected as his first post-Ph.D. professional employment, one which provided him an excellent opportunity of extending his knowledge into the Fungi Imperfecti. In 1941 he became Bibliographer and Assistant Curator of Fungi at the Farlow Herbarium under the late Dr. D. H. Linder. His principal energy was directed towards bringing Seymour's Host Index up to date. This particularly painstaking revision was completed in manuscript form up to 1942.

Dr. W. L. White examining mildewed fabric at the Quartermaster Tropical Deterioration Research Laboratory. 1947.

During this period he met Mary Rebecca Old, a graduate student in Biology at Radcliffe, and they were married in October 1942. "Becky" continued to work as an assistant in research to aid financially during the first years of their marriage.

With the inception of World War II the attention of scientific, industrial and military minds became sharply focused upon the

problems of tropical deterioration. What had been anticipated as a brilliant future in classical taxonomic mycology was diverted to mycological research in connection with the mildew-proofing of materiel with the Chemical Corps at Massachusetts Institute of Technology in 1943.

This was his first exposure to non-academic scientific life. The previous limitation of his activities to highly specialized taxonomic research revealed its eminent disadvantages in the strange environment. His approach and his personality did not fit into the alien schema of a highly practical, management-conscious organization. The demands for concurrent participation in multifold activities involving conflicting codes of behavior, rapid decisions based on approximations, mental resiliency and adaptations under compromising situations, impinged on unsuspecting Dr. White. His educational foundation provided neither the requisite points of departure, nor behavior models, nor "types" to which he could refer. Accordingly, when he went into military research with the Host Index unpublished, he was unable to carry on both activities at the same time. He could not switch back and forth from the immediate practical approximations of war-time deterioration problems to the detailed, meticulously accurate, patient completion of the Host Index. His compromise was to drop the Host Index. Viewed scientifically, this was unfortunate. Viewed practically, what other course was open to him?

Similar bifurcations confronted Dr. White when he became Chief of the Tropical Deterioration Research Laboratory in July 1944—a post he held for 4 years. Should he devote his energies to fundamental taxonomic research on the microorganisms themselves, or should he become involved in the practical and managerial demands of the situation? Recognizing his principal talents and envisioning that the war would soon be over, he wisely chose the basic research avenue. He adopted the singular purpose of setting up the Quartermaster Culture Collection on a firm taxonomic basis. The managerial, administrative and practical unpleasantries he would ignore and stoically bear the consequences. That his intuitive judgment was wise no one would deny today. The Collection soon became the focal point for much

and vigorous world-wide basic research in taxonomy, physiology and biochemistry. The cumulated data serve today as the basis for the practical development of mildew-proofing treatments for textiles, leather, paper, wood and other materials. Its cultures are the commercial standards used in annual procurement of millions of dollars worth of fungus-proofed materiel.

The four years exposure to this highly practical environment was not without impression upon his general outlook and thinking. His first organizational actions at the Farlow Herbarium and his later philosophy of taxonomic research provide an indication of this influence.

When Dr. White became Director of the Farlow Herbarium in April 1948, he was faced with the necessity of operating on funds the purchasing value of which had undergone a drastic decline. One of his first actions was to change the time-honored system of filing specimens. The phylogenetic or natural scheme was replaced by an alphabetical system, based on large groups. The distribution of specimens by mail was restricted. Boxes of unclassified material of unknown value were discarded. Only by such drastic actions was he able to hew out a bit of time for his own research.

During the war, Dr. White was deeply impressed with the eternal and awesome consequences of mistakes—mistakes that set up chains of irreversible decisions, acts and consequences. Corrections never seemed to catch up. This recognition moved him to adopt extreme caution in the field of his own specialty. His recent publications reveal a bringing together of information on organisms, specimens and records dealing with a particular species. But he rarely committed himself on the validity of names. He was never certain that he had *all* the evidence. As a specialist in several groups, he recognized the many misidentifications cluttering the literature. He hesitated to add another. As a mycologist of wide acquaintance, he was familiar with the abilities of his contemporaries. He knew that the reports of some were based on inadequate study and hasty publication. Wasn't it logical to assume that some of the "authorities" of the past were equally negligent? He viewed the straightening out of mycological tax-

onomy as a hopeless task, unless drastic action were taken. First, a halt must be called to the introduction of new names. Second, there must be a vigorous reworking of the entire taxonomic literature, and a weeding out of errors. He determined to set an example. One may demur that if the most skilful experts—and Dr. White certainly was a prominent one—do not set forth their views and estimates on new species, from whom will the less-informed learn? Yet Dr. White felt that drastic ailments require drastic remedies. He was willing to sacrifice his own publications for the cause.

Recognition came to Dr. White early. He served as vice president of the Mycological Society of America, and as Secretary and Chairman of the Microbiological Section of the Botanical Society of America. While with the Quartermaster Laboratory, he was invited by the Air Force to serve as mycologist on the round-the-world flight of the Air Technical Service Command Tropical Science Mission. The field experience gave him valuable first hand information on the extent and nature of deterioration of materiel by microorganisms. Recognition of White as the authority in this field is attested to by the fact that at the Farlow he was working on two projects for governmental agencies (Chemical Corps; Quartermaster Corps), and his assistance was being sought by three others.

This sketch brings us to early July 1952. Here was a most competent taxonomist on the threshold of his most productive period. Here was a world authority on the Helotiaceae and the Fungi Imperfecti, with a wealth of experience in the Basidiomycetes and Ascomycetes. Here was a friendly, democratic, helpful and competent professor. Here was a rare combination of the scientific and the practical, the academic and the managerial. Here was the synthesis of an essence to be handed down to the first of many graduate students and to be distilled for the scientific world. Then fate stepped in.

We who called him friend are thankful that his path crossed ours. His association has enriched our lives. His memory we cherish. We recall fondly the many incidents typifying the man —how he pounded his hand through a streetcar door when the

driver failed to stop—how his taste in food went to hamburger and potatoes—how he loved to stay up all night in discussion groups loudly putting forth his opinions. We remember that his struggle for an education was hard, that his success was largely due to his inherent goodness, his stubborn persistence, his intuitive taxonomic sense, and to those who loved him and helped him on. He needed no hobby; his enthusiasm and enjoyment were in his work and his family.

PIONEERING RESEARCH LABORATORIES,
U. S. ARMY QUARTERMASTER CORPS,
PHILADELPHIA, PENNSYLVANIA

PUBLICATIONS OF W. LAWRENCE WHITE

(arranged chronologically)

1. (with H. W. Thurston, Jr.). Notes on the Rusts of Pennsylvania. Proc. Penna. Acad. Sci. **7**: 140–148. 1933.
2. A new species of *Chondropodium* on *Pseudotsuga taxifolia*. Mycologia **28**: 433–438. 1936.
3. Note on *Conidiobolus*. Mycologia **29**: 148–149. 1937.
4. (with H. H. Whetzel). Pleomorphic life cycles in a new genus of the Helotiaceae. Mycologia **30**: 187–203. 1938.
5. (with H. H. Whetzel). *Mollisia tetrica*, *Peziza Sejourneí*, and the genera *Phaeociboria* and *Pycnopeziza*. Mycologia **32**: 609–620. 1940.
6. A Monograph of the genus *Rutstroemia* (Discomycetes). Lloydia **4**: 153–240. 1541. [Thesis-Cornell.]
7. A new Hemiascomycete. Canadian Jour. Res. **20(C)**: 389–395. 1942.
8. Studies in the genus *Helotium*. I. A review of the species described by Peck. Mycologia **34**: 154–179. 1942.
9. Studies in the genus *Helotium*. II. *Lachnum pygmaeum* and the status of the genus *Helolachnum*. Amer. Midl. Nat. **28**: 512–523. 1942.
10. Studies in the genus *Helotium*. III. History and diagnosis of certain European and North American foliicolous species. Farlowia **1**: 135–170. 1943.
11. Studies in the genus *Helotium*. IV. Some miscellaneous species. Farlowia **1**: 599–617. 1944.
12. (with R. Singer and W. H. Snell). The taxonomic position of *Polyporoletus sublividus*. Mycologia **37**: 124–128. 1945.
13. Deterioration of Quartermaster fabrics in the tropics. Quartermaster Rev. **26**: 16–17; 63–64; 67. 1946.
14. "Mycological factors," pp. 25–46 in Report of Army Air Forces Tropical Science Mission. Tropical Deterioration of Air Force Materiel and Equipment. Air Technical Service Command, Wright Field, Dayton, Ohio. 1946.

15. (with Mary H. Downing). The identity of "*Metarrhizium glutinosum*." Mycologia **39**: 546–555. 1947.
16. (with R. G. H. Siu). Resistance of resin-impregnated cotton fabrics to microorganisms. Ind. and Eng. Chem. **39**: 1628–1630. 1947.
17. (with R. T. Darby, Gladys M. Stechert, and Kathryn Sanderson). Assay of cellulolytic activity of fungi isolated from fabrics and related materials exposed in the tropics. Mycologia **40**: 34–84. 1948.
18. (with R. G. H. Siu and E. T. Reese). The black Aspergilli in relation to cellulosic substrata. Bull. Torrey Bot. Club **75**: 604–632. 1948.
19. (with C. C. Yeager and Helen Shotts). History, distribution and economic significance of the cellulose-destroying fungus *Memnoniella echinata*. Farlowia **3**: 399–423. 1949.
20. (with E. T. Reese, H. S. Levinson, and Mary H. Downing). Quartermaster Culture Collection. Farlowia **4**: 45–86. 1950.
21. (with G. R. Mandels and R. G. H. Siu). Fungi in relation to the degradation of woolen fabrics. Mycologia **42**: 199–223. 1950.
22. (with Mary H. Downing). *Coccospora agricola* Goddard, its specific status, relationships, and cellulolytic activity. Mycologia **43**: 645–657. 1951.
23. (with R. T. Darby). A refrigerator cabinet for fungal cultures. Mycologia **44**: 578–580. 1952.
24. (with Mary H. Downing). *Humicola grisea* Traaen, soil-inhabiting, cellulolytic Hyphomycete. Mycologia **45**. 1953 (in press).
25. (with G. D. Darker). Supplement to the Host Index of the Fungi of North America. (In manuscript; approx. 400 page book—unpublished.

Note: Government and University reports, abstracts, and book reviews have been omitted from the above list.

NOTES AND BRIEF ARTICLES

Synchytrium urticae

In a recent paper published shortly after his death Cook [1] described a new species of *Synchytrium* on *Urtica chamaedryoides* and named it *S. urticae*. This binomial, however, had been used previously by Sorokin [2] for a species which he found on *U. dioica* in Russia in 1873. It is surprising that Cook did not mention Sorokin's species because it was listed by Saccardo [3] and referred to as a questionable species by Tobler [4] in her monograph of *Synchytrium*.

Sorokin gave no measurements of his species but figured and described the resting spores as reniform, globose, oblong and elongate with a conspicuous pore and a densely verrucose or punctate, luminous, gray, fragile wall, as shown in the accompanying photograph of his drawings. According to his report the infected plants were recognizable from a distance by the yellow color of their leaves, which later dried up and shriveled. In the early stages of development of the parasite the infected epidermal cells do not differ from the neighboring healthy ones, but as the fungus matures the former cells become greatly enlarged. Nevertheless, they protrude only slightly above the surface of the adjacent epidermial cells, which apparently undergo little or no change. From Sorokin's figures it thus appears that his species produces only simple, unicellular galls. However, in herbarium material at the New York Botanical Garden which bears the name *S. urticae* and was distributed by Roumeguère (Fungi sel. exs. no. *1737*),

[1] Cook, M. T. 1952. Species of *Synchytrium* in Louisiana. VII. A new species on *Urtica chamaedryoides*. Mycologia **44**: 827–828.

[2] Sorokin, N. 1873. A few words about a new fungus, *Synchytrium urticae*. Contrib. 3rd Mtg. Russian Sci. Kiev, Bot. Trans., pp. 39–42, pl. 3. (Trans. from Russian.)

[3] Saccardo, P. A. 1888. Sylloge Fungorum **7**: 293.

[4] Tobler, G. 1912. Die Synchytrien. Studien zu einer Monographie der Gattung. Arch. protist'k. **28**: 141–238.

the author found numerous oval, 250 × 300 μ, elongate and almost spherical, 200–400 μ, composite, open and empty, protruding galls on the leaves of *Urtica dioica*.

Cook described his *S. urticae* as follows: "Galls abundant on stems, leaves, and flowers; more or less spherical, 48 × 48 to 93 × 156 μ. Sorus light yellow becoming dark yellow; 39 × 39 μ. Sporangia about 40 in number and 8–15 μ in diameter." Apparently no resting spores were found, and his observations concern only sporangial sori and sporangia.

However, in prepared slides of his species which Dr. Cook kindly sent me shortly before his death, the sori, sporangia and galls were found to be considerably larger than described by him. The sori were sub-spherical, 54.6–127.5 μ, or oval, 52–60 × 80–119 μ, and in several mature and ruptured ones 18 to 56 sub-spherical to spherical, 20–28 μ, oval, 17.4–20 × 34–43 μ, angular and polygonal sporangia were present. Galls were present on both sides of the leaves as well as on the stems, petioles and flower buds. They were composite, often compound, isolated or confluent, usually conspicuously protruding, predominantly sub-globular, hemispherical or sub-hemispherical, 180–244 × 300–720 μ, with an apical pore and a central canal extending down to the infected cell.

Whether or not Sorokin's and Cook's species are identical is open to question, but from Sorokin's description and figures they appear to be quite different. On the other hand, the galls on the material distributed by Roumeguère as *S. urticae* Sorokin on *U. dioica* are fundamentally similar to, but smaller than, those produced by Cook's species on *U. chamaedryoides*, and it is not improbable that Sorokin's brief description may be incomplete or incorrect. Equally plausible is the possibility that Roumeguère's material may relate to Cook's instead of to Sorokin's species. In this connection it is to be noted again that Sorokin found only resting spores, while Cook reported only sporangial sori and sporangia. Accordingly, the reported differences in gall structure and complexity produced by the two species may not preclude their being identical, because in some species, i.e., *S. cellularc*, the resting spores may produce simple, unicellular galls while the sporangial sori produce composite, multicellular galls on the same host. Ob-

viously, the problem of their identity cannot be solved until both species are fully known, but in the event Cook's species proves to be different and valid its name must be changed.—JOHN S. KARLING, Purdue University, Lafayette, Indiana.

Sorokin's plate of *Synchytrium urticae*. FIG. 1. Epidermal cells of *U. dioica* with a young parasite. FIG. 2. Mature *S. urticae*. FIG. 3. Profile of a cell with a resting spore of *S. urticae* n. sp. FIG. 4. Crushed spore of parasite. FIG. 5. Separated resting spores.

THE IDENTITY OF CORIOLOPSIS CROCATIFORMIS

In the examination of type material of polypores, it was found that some of the microscopic characters of *Coriolopsis crocatiformis* Murr. differed from the original description (N. Y. Bot. Gard. Bul. **8**: 139. 1912). Since microscopic details are now considered to be essential for the diagnosis of a species, the type collection, obtained from the New York Botanical Garden, was carefully re-examined.

The macroscopic features are as originally described (FIG. 1, A). The microscopic characters which were given as "spores sub-

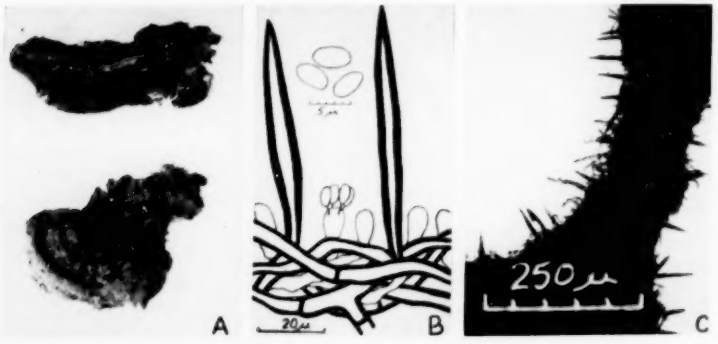

FIG. 1. *Coriolopsis crocatiformis* from type material. A. Upper surface and pore surface, × 1. B. Hymenium (much enlarged) showing the setae, basidia, spores and tramal hyphae. C. Cross section of the tubes showing the hymenium with numerous setae.

globose, smooth, hyaline, 3.5 μ; cystidia none" were not in entire agreement, for in 2 per cent KOH mounts it was found that the spores were cylindric-ellipsoid, 3.5–5 × 2–2.5 μ (FIG. 1, B), and that subulate setae 45–85 × 6–7 μ were present (FIG. 1, B, C). An emended description follows: Pileus sessile, spongy when dry, 1–2 × 1.5–4 × 0.2–0.5 cm.; surface yellowish-brown ("Ochraceous-Tawny" of Ridgway, "Color Standards and Color Nomenclature"), tomentose to spongy, velvety to touch, uneven, sulcate; margin thin, sterile below; context concolorous with the surface, soft and cottony, 0.1–0.2 cm. thick, the hyphae brown in KOH, thin- to thick-walled, occasionally septate, rarely branched, 2–4 μ in diame-

ter; tubes slightly darker than the context, 1–3 mm. long, the mouths irregular, becoming labyrinthiform by splitting of the walls of adjacent tubes, averaging 2 per mm., edges thin, somewhat lacerate to toothed, the tramal hyphae similar to the context hyphae except more frequently septated and branched; setae numerous, subulate, sometimes slightly enlarged at the base, thick-walled, 45–85 × 6–7 μ; basidia 9–14 × 4–5 μ; spores hyaline, smooth, apiculate, cylindric-ellipsoid, 3.5–5 × 2–2.5 μ.

The presence of setae relates this fungus to *C. sarcitiformis* Murr. which differs in having a radially fibrous upper surface, smaller pores, and shorter setae. *C. mellcoflava* Murr. (= *Irpex flavus* Kl. according to Bresadola in Ann. Myc. **14**: 232. 1916) slightly resembles this fungus but differs in having abundant capitate cystidia. No other species are known which invite comparison.

This fungus is known only from Belleville, Oaxaca, Mexico, and grateful acknowledgment is made to the New York Botanical Garden for the loan of the type collection. Thanks are also due to Dr. J. L. Lowe under whose direction this work was done.—JOHN HUNT, State University of New York College of Forestry, Syracuse, N. Y.

PENICILLIUM WITHIN OVULES OF ZAMIA

In connection with studies on fertilization and embryo development in *Zamia* which have been conducted by one of the authors (G. S. B.), shipments of living carpellate cones collected in Florida have been received at the University of Wisconsin Botany Department at intervals through the early weeks of the summer during each of the past few years. Many of the cones, representing *Z. umbrosa* Small, were collected near Gainesville by Dr. George Weber of the University of Florida, but numerous cones of another species (*Z. floridana* A. DC.) were obtained from the vicinity of Miami and from Dunnellon.

When, in 1950, some of the *Z. umbrosa* cones were dissected and their ovules opened, it was observed that a green mold was present in the nucellar cap area above the gametophyte. In other

instances no mold was visible but, as in the case of the ovules showing fungal spores, the nucellus was shrunken and abnormalities were evident in the upper part of the gametophyte. The latter was commonly slightly discolored or translucent and the archegonia were empty. In some cases mold was observed to fill the empty archegonia. A few gametophytes were also seen in which the micropylar end was considerably distorted and no archegonia had been formed. The ovules, however, usually appeared normal from the outside; only occasionally could a faint browning be detected on or through the integument at about the level of the nucellar cap.

From several surface-sterilized ovules aseptically cut open lengthwise, spores were removed to agar plates, and the fungus was thus easily brought into pure culture. Several ovules which showed no spores when opened but which exhibited discoloration of the gametophyte were placed in sterile moist chambers. These always yielded a crop of spores in the nucellar cap region within two or three days, and a green line of spores often followed the nucellus around the upper half of the megagametophyte. Hence it was concluded that mycelium probably was present throughout this entire portion of the nucellar tissue at the time of removal of the ovule from the strobilus. Bits of nucellus aseptically removed from ovules showing internal discoloration but no spores, quickly yielded mycelium and conidia when placed on the surface of nutrient agar in petri dishes. However, in no case was a mold growth obtained when aseptically-removed bits of discolored gametophyte were plated on agar, and in only one instance in several trials was a fungus recovered from suspected integument tissue similarly treated.

During July, 1950, approximately fifty isolations of the *Zamia* ovule mold were made. The isolates came from ovules removed from several different *Z. umbrosa* cones from two or three separate collections—all from the Gainesville area. In the summer of 1952 additional isolates were obtained from perhaps a dozen ovules, including some from *Z. floridana* strobili collected in the vicinity of Dunnellon. The same fungus was isolated in every case. Although no isolations from material originating in southern Florida were attempted, ovules exhibiting pathological features identical

with those described above were encountered in the Miami collections; thus it seems probable that the same fungus was present here also.

Study of the *Zamia* mold in plate culture on various media indicated that it belonged to the Asymmetrica-Velutina group of *Penicillium*, but a definite species determination could not be made. Cultures were then sent to Dr. K. B. Raper, who kindly examined them and reported that the fungus did not fit well into any of the described species. He suggested, however, that it might be considered an aberrant member of the *P. citrinum* series. Tests in the writers' laboratories, both on agar and in shaken-flask liquid cultures, have made clear that none of a half dozen randomly-selected isolates produces citrinin. That, together with other considerations, has led to tentative assignment of the fungus to a position near *P. corylophilum*.

Insofar as the authors can discover, this is the first report of the occurrence of *Penicillium* within the ovules of *Zamia;* but the frequency with which the organism was encountered in cones received from Florida during the past three years suggests that invasion of ovules must be common in that geographical area. Since the fungus usually is found in ovules showing no external damage and since the micropyle is sealed early in the development of the ovule, it would appear that the organism must gain entrance at the time of pollination. The fungus seems to show a strong preference for nucellar tissue and there is no evidence that it enters the gametophyte except to grow down into aborted archegonia. The observed modification of gametophyte tissue may result from diffusion of toxic material formed in the invaded nucellar cap area. The observations made suggest that the organism is pathogenic. Of considerable interest is the frequently heavy sporulation of the fungus deep within the ovule, where it would naturally be assumed that the oxygen supply is low.—M. P. BACKUS AND G. S. BRYAN (University of Wiscónsin).

CONCEPTS AND MISCONCEPTS IN TYMPANIS

The writer has recently read with great interest the paper by Dr. J. W. Groves (Canadian Journal of Botany **30**: 571–631. 1952) on the genus *Tympanis* in which he describes a number of

new species and supplies much added information on other species included by him in the genus. It is a valuable contribution but there is one statement to which the writer wishes to take exception, as might be expected. The statement is as follows: "*Seaver (1951) lists six species of Tympanis but apparently had a misconception of the genus.*" Since there are about as many interpretations of the genus as there are workers on the group who is to decide which of these is a misconception? Furthermore as knowledge increases concepts change. Let us point out some of these changes.

The writer began his studies on the Discomycetes more than fifty years ago, his objective being to list in one work the species occurring in North America so far as it is possible for one author to do so. In doing this it was necessary to deal with many genera and one could not help being impressed with the divergence of views regarding them and the changes of view as time went on and knowledge accumulated. We may use as an illustration the old genus *Peziza*. In the early days most all Discomycetes were placed in this genus. As the number increased, species and groups of species were removed and the original genus whittled down until today it includes only a small fraction of the species which it once comprised. Yet at this time it is doubtful if any two workers on this group would have exactly the same concept of the genus *Peziza* itself.

What has happened to *Peziza* has happened to other genera of the Discomycetes. This is especially true of *Tympanis*, the genus in question. In looking over the genera of the Cenangiaceae it will be noted that numerous species formerly placed in the genus *Tympanis* have been removed and relegated to other genera such as *Dermea, Pezicula, Godronia, Cenangella,* and *Dermatella,* largely on the basis of ascospore characters. As a result the genus *Tympanis* has also been reduced until it comprises only a very small percentage of the species originally included. But so far as the writer recalls, those species of Cenangiaceae in which the asci were filled with these minute spore-like bodies and in which the normal ascospores were either absent, undeveloped, or unobserved were retained in the genus and it came to be largely confined to those forms.

According to Groves normal ascospores, or what he terms primary ascospores, have been known in *Tympanis* since the time of Nylander (1868). Apparently they were not generally known for when William Phillips published his British Discomycetes in 1893, twenty-five years later, he listed eight species of *Tympanis*. In the six of these containing the minute spore-like bodies, normal ascospores were unknown or at least not known to him. The other two contained normal ascospores only and one of these had already been removed from the genus by Karsten. What became of the other the writer does not know. The point to be emphasized here is that at that early date the genus *Tympanis* had become restricted almost exclusively to those forms with the so-called secondary spores and in which the normal ascospores were unknown.

This is the concept (or misconcept) early adopted by the writer and hence *Tympanis* had come to be regarded as a form genus in which to place those species of Cenangiaceae in which the normal or primary ascospores were unknown. It was assumed that if and when true ascospores were found the species would be reclassified and placed in the proper genus as indicated by the character of the ascospores. There is a similar situation in the Hypocreales. A number of species in that order produced minute secondary ascospores which often obscured the normal ascospores. For some time these were kept together, but later distributed, according to the character of the ascospores, among the genera *Nectria, Calonectria, Scoleconectria* and *Thyronectria*.

To keep together in the same genus species with the diversity of ascospores reported by Dr. Groves for *Tympanis* would appear to the writer to be inconsistent with the treatment in other groups of Discomycetes. However this is not meant to be in any sense a criticism of the splendid work done by Dr. Groves on this and other genera of the Cenangiaceae but merely an attempt to explain and defend the concept of the genus *Tympanis* as presented by the writer in The North American Cup-fungi (Inoperculates), privately published. The writer claims no originality for this concept, neither does he feel inclined to retract or apologize for it. His views, however, are not binding on anyone else and all are

free to accept or reject as they may see fit.—FRED JAY SEAVER, Winter Park, Florida.

AN UNUSUALLY LARGE FOMES

Mycologists, like fishermen, like to report their largest catches. The giant puffball (*Calvatia maxima*) is certainly a leading contender for honors. Bessey, in his Textbook of Mycology (Blakiston's Son and Co. Phila. 1935), reports a sporophore measuring $1.6 \times 1.35 \times 0.24$ meters. In our local papers photographs of large fruiting bodies of *Polyporus berkeleyi* are to be seen most seasons. Just recently W. H. Wills (Mycologia **45**: 144–145, 1953) has reported such a specimen weighing 45 pounds and measuring up to 26 inches in diameter. A less familiar polypore, *Amauroderma brittonii*, is reported by Vélez (Turtox News **30**: 213, 1952) as measuring 90 cm. in diameter. Even the very abundant and well known *Fomes applantus* is to be listed among the species producing unusually large sporophores. Bessey (Textbook of Mycology) states that spore fruits of this species may attain a diameter of 75 cm. It is the purpose of this note to report an unusually large specimen of this species.

In November, 1942, while on a mycological class field trip, we happened upon a very large bracket of what was tentatively identified as *F. applanatus*. The bracket was attached to a living tree of *Acer rubrum* (red maple) growing in a bog forest near Aurora, Ohio. Using a small ruler the greatest diameter was determined to be 64 cm. This so impressed us that we photographed the specimen along with the 15 cm. rule (FIG. 1). The specimen was not collected, hence the tentative status of the identification. It was our hope that we could locate the bracket the following season and thereby follow its development. World War II interfered. Thanks to its location in a relatively inaccessible bog, no one had destroyed it during my long absence. Nature, however, was not so kind. Upon relocating the spot in July, 1948, we found that the host tree, as well as the perennial sporophore, had died. Decay had so weakened the bracket that it had partially fallen from the tree (FIG. 2). It was in such a condition as to discourage collection or further efforts of identification. We therefore con-

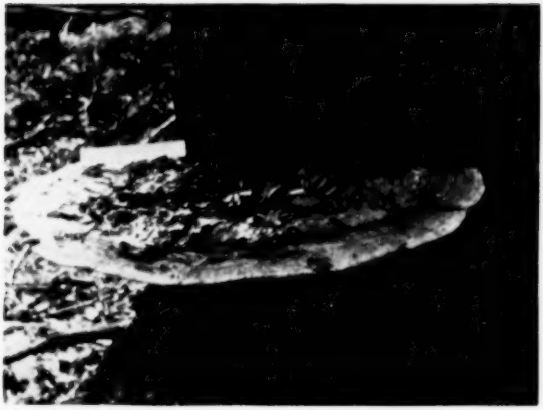

FIG. 1. Bracket of *Fomes applanatus*, with a 15 cm. rule, measuring 64 cm. from side to side. November, 1942. From a kodachrome.

tented ourselves with a new measurement and a new photograph. In order to determine the diameter it was necessary to assemble the fragments and then measure. Measuring the maximum side to side diameter, as in 1942, we found that it had increased from its former 64 cm. to a final measurement of 97 cm. As Vélez

FIG. 2. The same bracket as shown in Fig. 1 as seen in July, 1948, the maximum diameter, before breaking, having been 97 cm. From a kodachrome.

(above) says of his remarkable collection, "It is certainly one of the largest caps known."—J. ARTHUR HERRICK, Kent State University.

EIGHTH INTERNATIONAL CONGRESS OF BOTANY

The VIIIth International Congress will be held in Paris from Friday the 2nd to Wednesday the 14th of July, 1954. There will be a Post-Congress at Nice from July 22nd to the 25th or 26th. A number of excursions will be arranged before, during and after the Congress, including trips to north and equatorial Africa.

All who plan to attend should begin to make their plans. Notices have been sent to all those who may be supposed to be interested and whose names and addresses are available to the secretariat. Any who have not received announcements may secure them by writing to the Secretariat General du 8e Congress International de Botanique, 292, rue Saint-Martin, Paris 3e, France.

Dr. William Chambers Coker, known for his contributions to the knowledge of southeastern trees, in addition to his outstanding work on the fungi, died on June 27, in his eighty-first year.

REVIEWS

PHYTOPATHOLOGIE DES PAYS CHAUDS. Vol. II, by L. Roger (Encyclopedie Mycologique, Vol. XVIII). Pp. 1127–2256, figs. 154–361. Paul Lechevalier, Paris. 1953. Unbound. Price 10,000 francs (about $28.80).

The first volume of this ambitious work was reviewed in MYCOLOGIA **44**: 429. 1952. The second volume, of almost exactly the same size and even more profusely illustrated, covers diseases of tropical plants caused by Ascomycetes and imperfect fungi, arranged in the order of the causal fungi, following a rather conservative and familiar system of classification and with clear and usable keys.

Volume III, which is announced for publication this year, will include bacterial and virus diseases, diseases caused by algae, lichens, and parasitic flowering plants, a general discussion of disease control, arranged by hosts, and the indexes. The last will greatly facilitate the reference use of the work.

Considering the vast scope of the work and its necessarily limited circulation, the price is not excessive for these times, and the amount of information assembled and not readily available elsewhere makes it imperative that all laboratories where plant disease fungi are studied have it available.—G. W. M.

THE FUNGI. A DESCRIPTION OF THEIR MORPHOLOGICAL FEATURES AND EVOLUTIONARY DEVELOPMENT, by Ernst Albert Gäumann, translated by Frederick Lyle Wynd. 420 pp., 440 figs. Hafner Publishing Co., New York. 1952. Price $10.00.

The Gäumann text, published in Switzerland in 1949, needs no introduction to American mycologists. The appearance of an English translation will permit wider circulation and adds another to the group of excellent texts on the fungi now available in English.

The translator has followed the German text very closely, but

has rendered it into clear, idiomatic English. The most striking changes are in the bibliography, where the titles are given in full, and in the diagrams of life-cycles, which have been redrawn in a form clearer, to American readers at least, than in the original.

There are a few errors or ambiguities in translation, including those of the legends for Figs. 52, 203 and 242. The statement that "*Ascocorticium* produces a rind" (p. 229); that the endoperidium of *Astraeus* "lies free from the gleba" (p. 300) and the reference to the maturation of the gleba of *Hysterangium* (p. 306) are examples of faulty translation. The frequent reference to developmental trends within a group as evolutionary tendencies can be defended but perhaps over-emphasizes what is meant in the German original. These are relatively minor matters. It is well to have Gäumann's important book available to those to whom the original language is a barrier; for critical reference the original is readily available.—G. W. M.

PLANT DISEASES, by F. T. Brooks. Second edition. xii + 457 p., 62 figs. Oxford University Press, London, New York, Toronto. 1953. Price $7.50.

As indicated, this volume is a revision of a book published by Dr. Brooks in 1928. In general the arrangement is like that of the first edition: a brief introduction covering the general aspects as to cause of plant disease, their symptoms and their dissemination, their epidemic and sporadic occurrence, the host parasite relationships and environment, and ending with control and legislation on plant disease. Following this are chapters on nonparasitic and virus and bacterial diseases. Diseases caused by Actinomycetes and Plasmodiophorales precede the bulk of the discussion, which is given over to diseases caused by fungi arranged in mycological sequence. The book closes with a chapter on diseases caused by algae and one on fungicides. Excellently selected lists of references accompany each chapter.

The author has packed an immense amount of information into the available space and has shown good judgment in his selection of the material to be added to his earlier treatment.—JOSEPH C. GILMAN.

MANUSCRIPT

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